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ABSTRACT

This report on the health consequences of smokeless tobacco contains an "Introduction, Overview, and Conclusions" section and four major chapters. Chapter 1 defines the various forms of smokeless tobacco that are used in the United States and examines data pertaining to trends in prevalence and patterns of use. Methodological considerations are discussed and research needs are identified. Chapter 2 presents results of a systematic review of the world's medical literature describing experimental and human evidence pertinent to the evaluation of smokeless tobacco as a potential cause of cancer. Consensus summaries of the literature are presented in each of five categories: (1) epidemiological studies and case reports of oral cancer in relation to smokeless tobacco use; (2) epidemiological studies of other cancers in relation to smokeless tobacco; (3) chemical constituents, including carcinogens of smokeless tobacco; (4) metabolism of constituents of smokeless tobacco; and (5) experimental studies involving exposing laboratory animals to smokeless tobacco or its constituents. Chapter 3 addresses the health effects of smokeless tobacco use on the oral tissues through a systematic review of the relevant scientific literature of animal and human studies. Chapter 4 examines the consequences of exposure to nicotine from smokeless tobacco. The report concludes that the oral use of smokeless tobacco represents a significant health risk, can cause cancer and a number of noncancerous oral conditions, and can lead to nicotine addiction and dependence. (NB)

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The Health Consequences of Using Smokeless Tobacco

A Report of the Advisory Committee to the Surgeon General

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A Report of the
Advisory Committee to the Surgeon General

1986

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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FOREWORD

This report on *The Health Consequences of Using Smokeless Tobacco* completes the Public Health Service's initial examination of smokeless tobacco's role in the causation of cancer, noncancerous and precancerous oral diseases or conditions, addiction, and other adverse health effects. Almost 30 years after the Public Health Service's first statement on the health effects of cigarette smoking, it is now possible to issue the first comprehensive, indepth review of the relationship between smokeless tobacco use and health.

Ironically, while cigarette smoking has declined during the past 20 years, the production and apparent consumption of smokeless tobacco products have risen significantly. These increases are in marked contrast to the decline in smokeless tobacco use in the United States during the first half of this century. Indeed, smokeless tobacco products, particularly chewing tobacco and snuff, have recently emerged as popular products for the first time since the turn of the century. National estimates indicate that at least 12 million Americans used some form of smokeless tobacco during 1985 with use increasing especially among male adolescents and young male adults.

The increased use and appeal of this product assume major public health significance because the evidence reveals that smokeless tobacco can cause oral cancer, can lead to the development of oral leukoplakias and other oral conditions, and can cause addiction to nicotine. The strength of the association between these conditions and smokeless tobacco use combined with the upward trend in this behavior incites the same alarm as was true with the knowledge that spitting spread tuberculosis. That concern led to the original public rejection of tobacco chewing and dipping as unsanitary and antisocial. It is critical that our society prevent the use of this health hazard and avoid the tragic mistake of replacing the ashtray with the spittoon.

This report is the work of numerous experts within the Department of Health and Human Services and in the non-Federal scientific community. I express my gratitude for their contributions.

C. Everett Koop, M.D.
U.S. Surgeon General

PREFACE

This report discusses the health consequences of smokeless tobacco use. It constitutes a comprehensive review by an Advisory Committee to the Surgeon General of the available scientific literature to determine whether using smokeless tobacco increases the risk of cancer and noncancerous oral diseases and effects, leads to addiction and dependence, and contributes to other health consequences.

AFTER A CAREFUL EXAMINATION OF THE RELEVANT EPIDEMIOLOGIC, EXPERIMENTAL, AND CLINICAL DATA, THE COMMITTEE CONCLUDES THAT THE ORAL USE OF SMOKELESS TOBACCO REPRESENTS A SIGNIFICANT HEALTH RISK. IT IS NOT A SAFE SUBSTITUTE FOR SMOKING CIGARETTES. IT CAN CAUSE CANCER AND A NUMBER OF NONCANCEROUS ORAL CONDITIONS AND CAN LEAD TO NICOTINE ADDICTION AND DEPENDENCE.

The major overall conclusions of this report are the following:

1. It is estimated that smokeless tobacco was used by at least 12 million people in the United States in 1985 and that half of these were regular users. The use of smokeless tobacco, particularly moist snuff, is increasing, especially among male adolescents and young male adults.

2. The scientific evidence is strong that the use of snuff can cause cancer in humans. The evidence for causality is strongest for cancer of the oral cavity, wherein cancer may occur several times more frequently in snuff dippers compared to nontobacco users. The excess risk of cancer of the cheek and gum may reach nearly fiftyfold among long-term snuff users.

3. Some investigations suggest that the use of chewing tobacco may also increase the risk of oral cancer, but the evidence is not so strong and the risks have yet to be quantified.

4. Experimental investigations reveal potent carcinogens in smokeless tobacco. These include nitrosamines, polycyclic aromatic hydrocarbons, and radiation-emitting polonium. The tobacco-specific nitrosamines often have been detected at levels 100 or more times higher than Government-regulated levels of other nitrosamines permitted in foods eaten by Americans.

5. Smokeless tobacco use can lead to the development of oral leukoplakias (white patches or plaques of the oral mucosa), particularly at the site of tobacco placement. Based on evidence from several studies, a portion of leukoplakias can undergo transformation to dysplasia and further to cancer.
6. Gingival recession is a commonly reported outcome of smokeless tobacco use.
7. A number of studies have shown that nicotine exposure from smoking cigarettes can cause addiction in humans. In this regard, nicotine is similar to other addictive drugs such as morphine and cocaine. Since nicotine levels in the body resulting from smokeless tobacco use are similar in magnitude to nicotine levels from cigarette smoking, it is concluded that smokeless tobacco use also can be addictive. Besides, recent studies have shown that nicotine administered orally has the potential to produce a physiologic dependence.
8. Some evidence suggests that nicotine may play a contributory or supportive role in the pathogenesis of coronary artery and peripheral vascular disease, hypertension, peptic ulcers, and fetal mortality and morbidity.

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INTRODUCTION, OVERVIEW, AND CONCLUSIONS

DEVELOPMENT AND ORGANIZATION OF THE REPORT

This report from the Surgeon General's Advisory Committee on the Health Consequences of Using Smokeless Tobacco represents the first comprehensive assessment of the biomedical and behavioral literature describing experimental and human evidence on the health consequences of using smokeless tobacco. The content of this report is the work of numerous experts within the Department of Health and Human Services as well as distinguished scientists outside the organization.

Each chapter of the report was prepared based on manuscripts written by scientists who are recognized for their understanding of the specific content areas. Manuscripts were subjected to extensive peer review by a large number of experts in the specific areas of interest.

The report includes a "Preface" that presents the essence of the entire report and an "Introduction, Overview, and Conclusions." The body of the report consists of the following four chapters:

- Chapter 1—Prevalence and Trends of Smokeless Tobacco Use in the United States
- Chapter 2—Carcinogenesis Associated With Smokeless Tobacco Use
- Chapter 3—Noncancerous and Precancerous Oral Health Effects Associated With Smokeless Tobacco Use
- Chapter 4—Nicotine Exposure: Pharmacokinetics, Addiction, and Other Physiologic Effects

HISTORICAL PERSPECTIVE

The use of smokeless tobacco is a worldwide practice with numerous variations in the nature of the product used as well as in the customs associated with its use. In the United States, smokeless tobacco consists of chewing tobacco and snuff. The predominant mode of use of these nonsmoked tobaccos is oral, although they may be placed in or inhaled into the nasal cavity. Tobacco sniffing, however, has been and remains a rare practice in the United States.

Smokeless tobacco was used in the United States in the early 1600's when snuff made its way to the Jamestown Colony in Virginia through the efforts of John Rolfe in 1611 *(1)*. Evidence of tobacco chewing, however, was not found until a century later in 1704 *(2)*.

The use of tobacco, including smokeless tobacco, has been controversial since its introduction. In the past, tobacco use was considered by some as beneficial. As early as 3500 B.C., there are indications that tobacco was an article of established value to the inhabitants of Mexico and Peru. It appears that people who frequently lacked sufficient food alleviated their hunger pains by chewing tobacco *(3)*. Smokeless tobacco was also thought to have several medicinal uses. Among Native Americans, for example, chewing tobacco was used to alleviate toothaches, disinfect cuts, and relieve the effects of snake, spider, and insect bites *(4)*. Moreover, during the 19th and early 20th centuries in America, dental snuff was advertised to relieve toothache pain; to cure neuralgia, bleeding gums, and scurvy; and to preserve and whiten teeth and prevent decay *(1)*.

On the other hand, tobacco use historically has had numerous adversaries, including the following *(1)*:

- In 1590 in Japan, tobacco was prohibited. Users lost their property and were jailed.
- King James VI of Scotland in the early 1600's was a strong antismoking advocate who increased taxes on tobacco 4,000 percent in an attempt to reduce the quantity imported to England.
- In 1633, the Sultan Murad IV of Turkey made any use of tobacco a capital offense, punishable by death from hanging, beheading, or starvation. He maintained that tobacco caused infertility and reduced the fighting capabilities of his soldiers.
- The Russian Czar Michael Fedorovich, the first Romanov (1613–1645), prohibited the sale of tobacco, stating that users would be subjected to physical punishment and that persistent users would be killed.
- A Chinese law in 1683 threatened that anyone possessing tobacco would be beheaded.
- During the mid-1600's, Pope Urban VIII banned the use of snuff in churches, and Pope Innocent X attacked its use by priests in the Catholic Church.
- Other religious groups also banned snuff use: John Wesley, the founder of Methodism, attacked its use in Ireland; the Mormons, Seventh-Day Adventists, Parsees and Sikhs of India, Buddhist monks of Korea, members of the Tsai Li sect of China, and some Ethiopian Christian sects forbade the use of tobacco.

- Frederick the Great, King of Prussia, prevented his mother, the Dowager Queen of Prussia, from using snuff at his coronation in 1790.
- Louis XV, ruler of France from 1723 to 1774, banned snuff use from the Court of France.

Scientific observations concerning the health effects of smokeless tobacco use were first noted in 1761 by John Hill, a London physician and botanist who reported five cases of polypuses, a "swelling in the nostril that was hard, black and adherent with the symptoms of an open cancer" *(5)*. He concluded that nasal cancer could develop as a consequence of tobacco snuff use (sniffing).

Evidence that suggested a possible association between smokeless tobacco use and oral conditions in North America and Europe was not reported until 1915 when Abbe identified several tobacco chewers among a series of oral cancer patients and commented that smokeless tobacco use may be a risk factor for this cancer *(6)*. In the late 1930's, Ahblom observed in Sweden that more patients with buccal, gingival, and "mandibular" cancers than with other cancers reported the use of snuff or chewing tobacco *(7)*. In the United States, case reports of oral cancer among users of snuff or chewing tobacco appeared in the early 1940's *(8)*. The first epidemiologic study of smokeless tobacco was not conducted until the early 1950's *(9)*. Since that time, several scientists have described a pattern of increased risk of oral cancer among smokeless tobacco users.

Investigations of other possible health effects of smokeless tobacco use (e.g., noncancerous oral effects, addiction, and other physiologic consequences) are more recent subjects of scientific inquiry that have been undertaken primarily in the past two decades.

A brief review of the health consequences of smokeless tobacco was presented in the 1979 Surgeon General's report on smoking and health *(10)*. Since that review, the results of additional studies addressing the role of smokeless tobacco in health have become available and thus provide the basis of this current comprehensive review.

REVIEW METHODS

For the purpose of evaluating the scientific evidence to be included in this report, the Advisory Committee called upon the same criteria to determine causality as have been used for a number of Surgeon General's reports on smoking for the past two decades. The following criteria were used as the primary guidelines for assessing whether any associations between smokeless tobacco use and each of the disease areas or health conditions under examination were likely to be causal in nature:

- Consistency of the association—similar observations by multiple investigators in different locations and situations, at different times, and using different methods of study.
- Strength of the association—high ratio of disease rate for the population exposed to the suspected risk factor compared to the population unexposed to the risk factor.
- Specificity of the association—associations with the exposure exist for a specific or limited set of diseases, and associations with the disease exist for a specific or limited set of exposures.
- Temporal relationship of the association—exposure to the suspected etiologic factor precedes the disease.
- Coherence of the association—epidemiologic observations are consonant with all else that is known about the disease.

In addition to these criteria, the general principles employed by the International Agency for Research on Cancer (IARC)* in evaluating the carcinogenic risk of chemicals or complex mixtures (table 1) were used as needed to supplement the primary causation criteria *(11)*.

OVERVIEW

The use of smokeless tobacco products in the United States was widespread until the end of the 19th century. With the advent of antispitting laws, loss of social acceptability, and increased popularity of cigarette smoking, its use declined rapidly in this century. However, recent national data indicate a resurgence in smokeless tobacco habits with more than 12 million persons estimated as users of some form of smokeless tobacco in 1985. An upward trend in use is emerging, particularly among young males.

Given the evidence that smokeless tobacco is regaining popularity, serious questions have been raised about its adverse health effects. Most notably, this behavior has been linked to cancer, specifically, oral cancer. Analytic epidemiologic studies now indicate that the use of oral snuff increases the risk of oral cancer several fold and that among long-term snuff dippers the excess risk of cancers of the cheek and gum may reach nearly fiftyfold. This conclusion is consistent with the judgment of a recent working group of the IARC, which assessed the carcinogenic risk associated with tobacco habits other than smoking *(11)*.

The conclusion that smokeless tobacco causes cancer results from several lines of evidence: the presence of high levels of carcinogens in smokeless tobacco; the metabolic conversion of products of smokeless

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* The IARC was established in 1965 by the World Health Assembly as an independently financed organization within the framework of the World Health Organization. It conducts a program of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment.

TABLE 1.—General Principles in Evaluating Carcinogenic Risk of Chemicals or Complex Mixtures (International Agency for Research on Cancer)

- Evidence for carcinogenicity in experimental animals:
  - — Qualitative aspects:
    - (a) Experimental parameters under which chemical was tested.
    - (b) Consistency with which chemical shown to be carcinogenic.
    - (c) Spectrum of neoplastic response.
    - (d) Stage of tumor formation in which chemical involved.
    - (e) Role of modifying factors.
  - — Hormonal carcinogenesis.
  - — Complex mixtures.
  - — Quantitative aspects; increasing incidence of neoplasms with increasing exposure.
- Evidence for activity in short-term tests:
  - — Use of valid test system.
  - — Sufficiently wide dose range and duration of exposure to the agent and appropriate metabolic system employed in test.
  - — Use of appropriate controls.
  - — Specification of the purity of the compound, and in the case of complex mixtures, source and representativeness of sample tested.
- Evidence of carcinogenicity in humans:
  - — For studies showing positive association:
    - (a) Existence of no identifiable bias.
    - (b) Possibility of positive confounding considered.
    - (c) Association unlikely to be due to chance alone.
    - (d) Association is strong.
    - (e) Existence of dose-response relationship.
  - — For studies showing no association:
    - (a) Existence of no identifiable negative bias.
    - (b) Possibility of negative confounding considered.
    - (c) Possible effects of misclassification of exposure or outcome have been weighed.

tobacco into genotoxic agents; the consistency of the oral cancer-smokeless tobacco association across epidemiologic investigations conducted in diverse locations; the trend in increasing oral cancer risk with duration of exposure; the strength of the association with oral cancer; and the occurrence of the highest risks for cancers at the anatomic sites where the tobacco exposures are the greatest.

In addition, a number of clinical observations and studies show an association between smokeless tobacco use and some noncancerous and precancerous oral health conditions. The development of a portion of oral leukoplakias in both teenage and adult users can be attributed to the use of smokeless tobacco. The risk of developing these leukoplakic lesions increases with increased exposure, and a number of studies now suggest that some snuff-induced leukoplakias can undergo transformation to dysplasia and further to carcinoma. The evidence concerning the adverse health effects of smokeless tobacco use on other oral soft and hard tissues is only suggestive at this time.

The magnitude of blood nicotine levels resulting from smokeless tobacco use has been shown to be similar to that from cigarette smoking. Therefore, the nicotine-related health consequences of smoking would also be expected to result from smokeless tobacco use. Given the nicotine content of smokeless tobacco, the user's ability to sustain elevated blood levels of nicotine, and the well-established data implicating nicotine as an addictive substance, it is reasonable to expect that smokeless tobacco is capable of producing nicotine addiction in users.

There is also some suggestive evidence that nicotine may play a contributory or supportive role in the development of coronary artery and peripheral vascular disease, hypertension, peptic ulcer disease, and fetal mortality and morbidity.

The conclusions in this report on the relationship between smokeless tobacco use and cancer, noncancerous and precancerous oral conditions, and addiction and dependence are substantially in agreement with those published at a recent National Institutes of Health (NIH) Consensus Development Conference on the Health Implications of Smokeless Tobacco Use *(12)*.

CONCLUSIONS

Prevalence and Trends of Smokeless Tobacco Use in the United States

1. Recent national data indicate that over 12 million persons used some form of smokeless tobacco (chewing tobacco and snuff) in 1985 and that approximately 6 million used smokeless tobacco weekly or more often. Use is increasing, particularly among young males.

2. The highest rates of use are seen among teenage and young adult males. A recent national survey indicates that 16 percent of males between 12 and 25 years of age have used some form of smokeless tobacco within the past year and that from one-third to one-half of these used smokeless tobacco at least once a week. Use by females of all ages is consistently less than that of males; about 2 percent have used smokeless tobacco in the last year.

3. State and local studies corroborate the national survey findings. The prevalence of smokeless tobacco use by youth and young adults varies widely by region, but use is not limited to a single region. In several parts of the country, as many as 25 to 35 percent of adolescent males have indicated current use of smokeless tobacco.

Carcinogenesis Associated With Smokeless Tobacco Use

1. The scientific evidence is strong that the use of smokeless tobacco can cause cancer in humans. The association between smokeless tobacco use and cancer is strongest for cancers of the oral cavity.
2. Oral cancer has been shown to occur several times more frequently among snuff dippers than among nontobacco users, and the excess risk of cancers of the cheek and gum may reach nearly fiftyfold among long-term snuff users.
3. Some investigations suggest that the use of chewing tobacco also may increase the risk of oral cancer.
4. Evidence for an association between smokeless tobacco use and cancers outside of the oral cavity in humans is sparse. Some investigations suggest that smokeless tobacco users may face increased risks of tumors of the upper aerodigestive tract, but results are currently inconclusive.
5. Experimental investigations have revealed potent carcinogens in snuff and chewing tobacco. These include nitrosamines, polycyclic aromatic hydrocarbons, and radiation-emitting polonium. The tobacco-specific nitrosamines N-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone have been detected in smokeless tobacco at levels 100 times higher than the regulated levels of other nitrosamines found in bacon, beer, and other foods. Animals exposed to these tobacco-specific nitrosamines, at levels approximating those thought to be accumulated during a human lifetime by daily smokeless tobacco users, have developed an excess of a variety of tumors. The nitrosamines can be metabolized by target tissues to compounds that can modify cellular genetic material.
6. Bioassays exposing animals to smokeless tobacco, however, have generally shown little or no increased tumor production, although some bioassays suggest that snuff may cause oral tumors when tested in animals that are infected with herpes simplex virus.

Noncancerous and Precancerous Oral Health Effects Associated With Smokeless Tobacco Use

1. Smokeless tobacco use is responsible for the development of a portion of oral leukoplakias in both teenage and adult users. The degree to which the use of smokeless tobacco affects the oral hard and soft tissues is variable depending on the site of action, type of smokeless tobacco product used, frequency and duration of use, predisposing factors, cofactors (such as smoking or concomitant gingival disease), and other factors not yet determined.

2. Dose response effects have been noted by a number of investigators. Longer use of smokeless tobacco results in a higher prevalence of leukoplakic lesions. Oral leukoplakias are commonly found at the site of tobacco placement.

3. Some snuff-induced oral leukoplakic lesions have been noted upon continued smokeless tobacco use to undergo transformation to a dysplastic state. A portion of these dysplastic lesions can further develop into carcinomas of either a verrucous or squamous cell variety.

4. Recent studies of the effects of smokeless tobacco use on gingival and periodontal tissues have resulted in equivocal findings. While gingival recession is a common outcome from use, gingivitis may or may not occur. Because longitudinal data are not available, the role of smokeless tobacco in the development and progression of gingivitis or periodontitis has not been confirmed.

5. The evidence concerning the effects of smokeless tobacco use on the salivary glands is inconclusive.

6. Negative health effects on the teeth from smokeless tobacco use are suspected but unconfirmed. Present evidence, albeit sparse, suggests that the combination of smokeless tobacco use in individuals with existing gingivitis may increase the prevalence of dental caries compared with nonusers without concomitant gingivitis. Reports of tooth abrasion or staining have not been substantiated through controlled studies; only case reports are available.

Nicotine Exposure: Pharmacokinetics, Addiction, and Other Physiologic Effects

1. The use of smokeless tobacco products can lead to nicotine dependence or addiction.

2. An examination of the pharmacokinetics of nicotine (i.e., nicotine absorption, distribution, and elimination) resulting from smoking and smokeless tobacco use indicates that the magnitude of nicotine exposure is similar for both.

3. Despite the complexities of tobacco smoke self-administration, systematic analysis has confirmed that the resulting addiction is similar to that produced and maintained by other addictive drugs in both humans and animals. Animals can learn to discriminate nicotine from other substances because of its effects on the central nervous system. These effects are related to the dose and rate of administration, as is also the case with other drugs of abuse.

4. It has been shown that nicotine functions as a reinforcer under a variety of conditions. It has been confirmed that nicotine can

function in all of the capacities that characterize a drug with a liability to widespread abuse. Additionally, as is the case with most other drugs of abuse, nicotine produces effects in the user that are considered desirable to the user. These effects are caused by the nicotine and not simply by the vehicle of delivery (tobacco or tobacco smoke).

5. Nicotine is similar in all critical measures to prototypic drugs of abuse such as morphine and cocaine. The methods and criteria used to establish these similarities are identical to those used for other drugs suspected of having the potential to produce abuse and physiologic dependence. Specifically, nicotine is psychoactive, producing transient dose-related changes in mood and feeling. It is a euphoriant that produces dose-related increases in scores on standard measures of euphoria. It is a reinforcer (or reward) in both human and animal intravenous self-administration paradigms, functioning as do other drugs of abuse. Additionally, nicotine through smoking produces the same effects, and it causes neuroadaptation leading to tolerance and physiologic dependence. Taken together, these results confirm the hypothesis that the role of nicotine in the compulsive use of tobacco is the same as the role of morphine in the compulsive use of opium derivatives or of cocaine in the compulsive use of coca derivatives.
6. The evidence that smokeless tobacco is addicting includes the pharmacologic role of nicotine dose in regulating tobacco intake; the commonalities between nicotine and other prototypic dependence-producing substances; the abuse liability and dependence potential of nicotine; and the direct, albeit limited at present, evidence that orally delivered nicotine retains the characteristics of an addictive drug.
7. Several other characteristics of tobacco products in general, including smokeless tobacco, may function to enhance further the number of persons who are afflicted by nicotine dependence: nicotine-delivering products are widely available and relatively inexpensive; and the self-administration of such products is legal, relatively well tolerated by society, and produces minimal disruption to cognitive and behavioral performance. Nicotine produces a variety of individual-specific therapeutic actions such as mood and performance enhancement; and the brief effects of nicotine ensure that conditioning occurs, because the behavior is associated with numerous concomitant environmental stimuli.
8. All commonly marketed and consumed smokeless tobacco products contain substantial quantities of nicotine. The nicotine is delivered to the central nervous system in addicting quantities when used in the fashion that each form is commonly used (or as recommended in smokeless tobacco marketing campaigns).

9. Since the exposure to nicotine from smokeless tobacco is similar in magnitude to nicotine exposure from cigarette smoking, the health consequences of smoking that are caused by nicotine also would be expected to be hazards of smokeless tobacco use. Areas of particular concern in which nicotine may play a contributory or supportive role in the pathogenesis of disease include coronary artery and peripheral vascular disease, hypertension, peptic ulcer disease, and fetal mortality and morbidity.

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Chapter 1.

PREVALENCE AND TRENDS OF SMOKELESS TOBACCO USE IN THE UNITED STATES

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INTRODUCTION

This chapter defines the various forms of smokeless tobacco that are used in the United States and examines the data that pertain to trends in prevalence and patterns of use. Trends in smokeless tobacco production and sales and self-reported use are considered. Methodological considerations are discussed and research needs are identified.

Tobacco was used by pre-Columbian American Indians in smokeless forms as well as smoked *(1)*. Cultivated by American colonists, tobacco became a major commodity in trade with Europe. Until the end of the 19th century, the use of smokeless tobacco products was widespread in the United States. Its use declined rapidly in this century with the advent of antispitting laws, loss of social acceptability, and increased popularity of cigarette smoking *(1,2)*. Use was primarily confined to rural and agricultural areas and to occupational settings where smoking was not allowed, such as mining and some industries *(3,4)*. In the Southeastern United States, especially in rural areas, oral use of dry snuff remained popular among women *(5,6)*.

PRODUCT CHARACTERISTICS

Today, smokeless tobacco is produced in two general forms: chewing tobacco and snuff *(7-10)*. Chewing tobacco is chewed or held in the cheek or lower lip. Three primary types of chewing tobacco are marketed: looseleaf, plug, and twist. Snuff has a much finer consistency than chewing tobacco and is held in place in the mouth without chewing. It is marketed in both dry and moist forms. Although smokeless tobacco is not subject to combustion and is usually used orally in the United States, products differ with regard to several factors, including type of tobacco plant used, parts of the tobacco plant used, method of curing, moisture content, and additives. For example, looseleaf chewing tobacco is made from air-cured, cigar-type leaves from tobacco that is grown in Pennsylvania and Wisconsin. In contrast, dry snuff is made primarily from fire-cured dark tobacco that is grown in Kentucky and Tennessee. Plug tobacco and snuff come in dry and moist forms. Many smokeless tobacco products are sweetened with sugar or molasses. Many are flavored; licorice is a common additive for chewing tobacco, while mint and wintergreen often are used to flavor snuff. Table 1 describes the types of smokeless tobacco and how they are used and packaged *(7-10)*.

TRENDS IN PRODUCTION AND SALES

United States Department of Agriculture (USDA) records on the annual production and sales of smokeless tobacco serve as indicators of the population's consumption. Changes in consumption can be inferred from changes in production and sales. Because sales figures closely resemble those for production, only production will be reported.

TABLE 1.—Characteristics of Smokeless Tobacco Products

Product	Description	How Used	Packaging*
CHEWING TOBACCO			
Looseleaf	Made from air-cured, cigar leaf tobaccos of Pennsylvania and Wisconsin. Consists of stripped and processed tobacco leaves. The leaves are stemmed, cut, or granulated and are loosely packed to form small strips of shredded tobacco. Most brands are sweetened and flavored with licorice.	A piece of tobacco, 3/4 to 1 inch in diameter, is tucked between the gum and jaw, usually to the back of the mouth.	Pouch, typically 3 ounces. A few brands market a 1.5-ounce pouch.
Plug	Made from enriched tobacco leaves (Burley and bright tobacco and cigar tobacco) or fragments wrapped in fine tobacco and pressed into bricks. May be firm (less than 15 percent moisture) or moist (15 percent or greater moisture). Most plug tobacco is sweetened and flavored with licorice.	Chewed or held in the cheek or lower lip. May be held in the mouth for several hours.	A compressed brick or flat block wrapped inside natural tobacco leaves. Packaged in clear plastic. Packages range from 7 to 13 ounces. Also sold by the piece.
Twist	Handmade of dark, air-cured leaf tobacco treated with a tarlike tobacco leaf extract and twisted into strands that are dried. Majority is sold without flavoring and sweeteners.	Similar to plug.	A pliable but dry rope. Sold by the piece, packaged in plastic bags. No standard weight. Sold in small (approximately 1-2 ounces) and larger sizes based on the number of leaves in the twist.
SNUFF			
Moist	Made from air-cured and fire-cured tobacco. Consists of tobacco stems and leaves that are processed into fine particles or strips. Some products are flavored. Has a moisture content of up to 50 percent.	A small amount ("pinch") is placed between the lip or cheek and gum and is typically held for 30 minutes or longer per pinch.	Cans and plastic containers, typically 1.2 ounces.
Dry	Most dry snuff is made from fire-cured tobaccos of Kentucky and Tennessee. After initial curing, the tobacco is fermented further and processed into a dry powdered form. Products vary in strength and flavoring. Generally has a moisture content of less than 10 percent.	Same as moist snuff. May also be sniffed.	Metal cans or glass containers, vary from 1.15 to 7 ounces per container.

* Product weight (includes moisture).

Categories of Products

The USDA reports production and sales by product category (i.e., chewing tobacco and snuff). The definitions of categories changed in 1981. Prior to 1981, total figures for chewing tobacco were derived by summing data for the subcategories of plug, twist, looseleaf, and fine-cut; snuff was a separate category. However, fine-cut tobacco is used in moist snuff. To reflect this fact, after 1981 USDA shifted fine-cut from the category of chewing tobacco to moist snuff. To observe and clarify temporal trends for the purposes of this review, the data presented in figure 1 reflect a uniform category system across years. In these records, fine-cut tobacco is counted consistently as snuff *(11-17)*.

Temporal Trends

Figure 1 depicts temporal trends in the quantities of smokeless tobacco that were manufactured in the United States from 1961 to 1985. Between 1944 and 1968, total smokeless tobacco production declined 38.4 percent from 150.2 to 92.5 million pounds. Subsequent increases in production reached 135.6 million pounds in 1985.

Between 1970 and 1985, total snuff production increased 56 percent from 31.3 to 48.7 million pounds. This increase was due to changes in the production of moist snuff; the manufacture of dry snuff declined *(3)*. The difference in trends in the production of moist and dry snuff is shown in figure 1 for the years 1981 through 1985. Separate production data are not available for the two types of snuff prior to 1981. Between 1970 and 1981, however, the production of fine-cut tobacco, used in the manufacture of some moist snuff, increased threefold from 4.8 to 15.2 million pounds.

Between 1970 and 1985, the production of chewing tobacco increased 36 percent from 63.9 to 86.9 million pounds. This increase was due to the production of looseleaf tobacco, which increased 87.3 percent from 39.5 to 74.0 million pounds. The production of plug and twist tobacco declined during this period.

TRENDS IN SELF-REPORTED USE: SURVEY DATA

National Survey Data

National data from 1964 to 1985 are available from eight different national probability surveys and a national survey of college students. The majority of the data pertain to persons over the age of 17. The principal characteristics of these surveys are shown in table 2.

Office on Smoking and Health Surveys

Early data on the use of chewing tobacco and snuff are available from the 1964, 1966, 1970, and 1975 Adult Use of Tobacco Surveys that were

FIGURE 1.—Manufacturing Trends: Quantities of Smokeless Tobacco Manufactured in the United States From 1961 to 1985 Expressed in Million Pounds

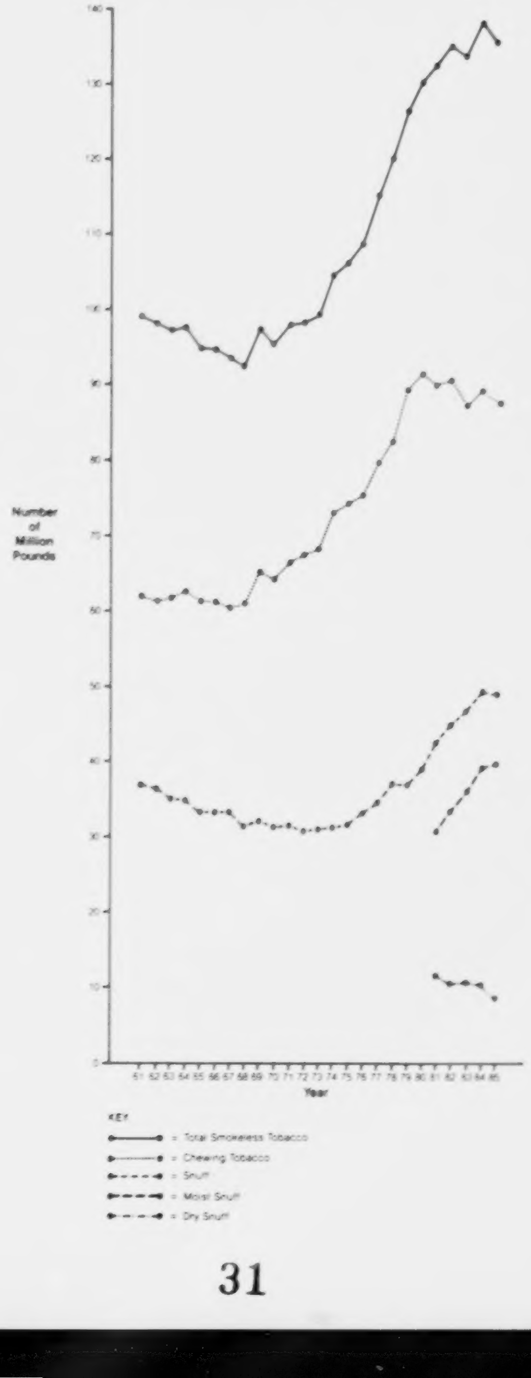

TABLE 2.—National Prevalence of Smokeless Tobacco Use: Data Sources

Survey	Type	Date	Respondents	Number of Respondents/ Households	Products	Questions
Office on Smoking and Health	Personal Interview	1964	Adults ≥ 21	5,794	Snuff and Chewing Tobacco Separately	"Have you ever used—at all regularly?" "Do you use—now?"
Office on Smoking and Health	Personal Interview	1966	Adults ≥ 21	5,770	Snuff and Chewing Tobacco Separately	"Have you ever used—at all regularly?" "Do you use—now?"
Office on Smoking and Health	Telephone	1970	Adults ≥ 21	5,200	Snuff and Chewing Tobacco Separately	"Have you ever used—at all regularly?" "Do you use—now?"
Office on Smoking and Health	Telephone	1975	Adults ≥ 21	12,000	Snuff and Chewing Tobacco Separately	"Have you ever used—at all regularly?" "Do you use—now?"
National Health Interview Survey Supplement (National Center for Health Statistics)	Personal Interview Including Proxy	1970	Persons ≥ 17	77,000/ 37,000	Snuff and Chewing Tobacco Separately	Does ________ presently use any other form of smokeless tobacco, such as snuff or chewing tobacco?
Simmons Study of Media Markets, Simmons Market Research Bureau, Inc.	Questionnaire	1980 1981 1982 1983 1984 1985	Adults ≥ 18	15,000- 19,000	Snuff Only	1980 to 1983 "Do you use it yourself—snuff (smokeless tobacco)?" 1984 to 1985 "Do you yourself use any of the following tobacco products?" Snuff (ST) listed as an option.
Simmons National College Study, Simmons Market Research Bureau, Inc.	Questionnaire	1983 1985	College Students ≥ 18	2,011- 2,373	Snuff Only	"Please mark which of the items listed below you yourself use." Snuff (smokeless tobacco) listed as an option.
Current Population Survey Supplement—Census Bureau for Office on Smoking and Health	Personal Interview Including Proxy	1985	Persons ≥ 16	120,000/ 58,000	Snuff and Chewing Tobacco Separately	Does ________ presently use any other form of tobacco, such as snuff or chewing tobacco? What other forms of tobacco does ________ presently use?
NIDA Household Survey	Personal Interview	1985	Persons ≥ 12	8,000	Snuff and Chewing Tobacco Combined	"On the average, in the past 12 months, how often have you used chewing tobacco or snuff or other smokeless tobacco?"

TABLE 3.—Use of Smokeless Tobacco in the United States by Individuals Over 21 Years of Age*

	Percentage of Users							
	Males				Females			
Use Category	1964	1966	1970	1975	1964	1966	1970	1975
Now Use Snuff	2.0	3.1	2.9	2.5	2.0	2.1	1.4	1.3
Used to Use Snuff	3.6	3.9	4.2	4.0	0.9	1.0	1.1	1.1
Have Ever Used Snuff †	5.7	7.2	7.1	6.4	2.9	3.1	2.6	2.4
Now Use Chewing Tobacco	5.1	7.1	5.6	4.9	0.5	0.4	0.6	0.6
Used to Use Chewing Tobacco	12.0	13.2	19.1	16.1	1.0	1.1	1.8	1.2
Have Ever Used Chewing Tobacco†	17.2	20.5	24.7	21.0	1.5	1.5	2.4	1.8

* "Use" not further defined with respect to frequency.

† Includes those who used to use, but did not state if they used it currently.

Source: National Clearinghouse on Smoking and Health.

conducted by the National Clearinghouse for Smoking and Health, currently the Office on Smoking and Health (OSH) *(18-20)*. National probability samples of 5,700 to 12,000 individuals over the age of 21 from randomly selected households were interviewed by telephone regarding the use of tobacco products. Between 1964 and 1975, the prevalence of smokeless tobacco use remained fairly stable. Results are summarized in table 3. Three patterns in these data may be noted:

- Less than 5 percent of the population reported using smokeless tobacco.
- Nationally, use was higher among males than females.
- Among males, the prevalence of use of chewing tobacco was higher than that for snuff.

National Health Interview Survey

In 1970, the National Center for Health Statistics included a question on current use of snuff and chewing tobacco in its National Health Interview Survey (NHIS) *(21)*. One respondent per household provided information on all household members age 17 and older. Data were collected on approximately 77,000 persons in 37,000 households. Estimates indicate that 1.4 percent of males used snuff and 3.8 percent used chewing tobacco (table 4).

Simmons Market Research Bureau, Inc.

National probability data that were collected annually from 1980 through 1985 for the Simmons Study of Media and Markets provide estimates of the prevalence of snuff use among adults who were 18 years of age or older. Sample size ranged from 15,000 to 19,000. Data are summarized in table 5 for the years 1980 to 1985. The prevalence

TABLE 4.—Prevalence of the Use of Snuff and Chewing Tobacco Among Males by Age, 1970 NHIS and 1985 CPS Surveys*

	1970 HIS		1985 CPS	
Product	Age	Percentage of Users	Age	Percentage of Users
Snuff	17-19	0.3	16-19	2.9
	20-29	0.6	20-29	2.7
	30-39	0.7	30-39	1.8
	40-49	1.2	40-49	1.5
	50+	2.7	50+	1.4
	Total	1.4	Total	1.9
Chewing Tobacco	17-19	1.2	16-19	3.0
	20-29	1.9	20-29	4.2
	30-39	2.8	30-39	3.7
	40-49	3.0	40-49	3.3
	50+	6.5	50+	4.2
	Total	3.8	Total	3.9

* "Use" not further defined with respect to frequency.

Sources: National Center for Health Statistics, National Health Interview Survey, 1970 (unpublished). Office on Smoking and Health, Current Population Survey, 1985 (unpublished).

TABLE 5.—National Prevalence of Current Use of Snuff by Gender, Age, and Race for 1980 Through 1985*

	Percentage of Users					
Sample	1980	1981	1982	1983	1984	1985
Total	1.6	2.2	2.6	2.3	1.9	1.9
Gender						
Males	2.4	3.7	4.2	3.8	3.0	3.2
Females	0.8	0.8	1.1	0.9	1.0	0.7
Age						
18-24	1.4	2.6	4.3	3.5	3.2	2.8
25-34	2.5	2.8	3.1	3.0	2.0	2.1
35-44	1.0†	1.3	1.6	1.8	1.5	1.0
45-54	1.3†	1.3	1.4†	1.0†	1.1†	1.5
55-64	1.2†	1.7	1.7	2.3	1.1†	1.3
≥ 65	1.6†	2.8	2.6	1.4	2.5	2.4
Race						
Black	2.3†	1.6†	3.0	2.9	2.9	2.4
White	1.5	2.2	2.6	2.3	1.9	1.9
Other	1.9†	1.4†	1.1†	NA	0.4†	1.2

* Adults defined as individuals over 18 years of age. Use not further defined with respect to frequency.

† Number of cases too small for reliable estimates.

Source: Simmons Market Research Bureau, Inc., Study of Media and Markets, 1980-1985.

TABLE 6.—Prevalence of Snuff Use Among College Students 18 Years of Age or Older by Gender and Year*

Sample	Percentage of Users	
	1983	1985
Total	2.7	3.5
Gender		
Males	5.4	6.7
Females	0.1†	0.2†
Race		
Black	1.5†	1.4†
White	5.1	3.6
Other	4.9†	4.3†

* Current use; frequency of use not specified.

† Projection relatively unstable because of small sample.

Source: Simmons Market Research Bureau, Inc., Simmons National College Study, 1983 and 1985.

rate for "current use" of snuff was 2.4 percent for males in 1980 and 0.8 percent for females. Rates for males peaked at 4.2 percent in 1982 and were 3.2 percent in 1985. Since 1982, the highest rates of use have consistently been observed in the age group 18 to 24 years old. Comparatively higher rates of use were also observed in the age groups 25 to 34 years old and over age 65 *(22)*.

The Simmons National College Study reports data from a probability sample of full-time students 18 years or older who were attending baccalaureate-granting colleges and universities in the coterminous United States. In 1983, 2,011 students were sampled, and 2,373 students were sampled in 1985. Five to 7 percent of males indicated use of snuff compared to 0.2 percent of females (table 6). The prevalence rate among male students exceeded that of the general adult male population (tables 5 and 6). In 1985, prevalence among college males was twice that of other adult males, while the rate for college women was less than one-third that among the general adult female population. The combined prevalence for male and female college students (3.5 percent) was very similar to that for 18- to 24-year-olds in the general population (2.8 percent) (tables 5 and 6) *(23)*.

Current Population Survey

In the fall of 1985, the Census Bureau collected health information on approximately 120,000 persons in 58,000 households in its Current Population Survey (CPS) *(24)*. OSH sponsored a supplement to this survey, which included a question on current use of snuff and chewing tobacco. One respondent per household provided information on all members age 16 and older. Provisional estimates of smokeless tobacco use indicate that 1.9 percent of males used snuff and 3.9 percent used chewing tobacco (table 4).

TABLE 7.—National Prevalence of Smokeless Tobacco Use by Adult Status and Sex, NIDA Sample, 1985*

	Percentage of Users			
	Males		Females	
Use Category	≤ 20 Years	≥ 21 Years	≤ 20 Years	≥ 21 Years
Used in Past Year	16	11	2	2
Used Formerly	4	7	2	2
Never Used	79	82	96	96

* Preliminary estimates not adjusted for oversampling of blacks and Hispanics.

Source: National Institute on Drug Abuse, 1985 National Household Survey on Drug Abuse. Preliminary results presented at the NIH Consensus Development Conference on the Health Implications of Smokeless Tobacco Use, January 1986.

TABLE 8.—Recency of Smokeless Tobacco Use by Sex and Age Group*

	Percentage of Users by Age Groups							
	12-17		18-25		26-39		40 +	
Use Category	Males	Females	Males	Females	Males	Females	Males	Females
Used in Past Year	16	1	16	1	10	1	8	3
Used Formerly	4	2	7	1	5	1	8	2
Never Used	80	97	77	98	85	98	84	95

* Preliminary estimates not adjusted for oversampling of blacks and Hispanics.

Source: National Institute on Drug Abuse, 1985 National Household Survey on Drug Abuse. Preliminary results presented at the NIH Consensus Development Conference on the Health Implications of Smokeless Tobacco Use, January 1986.

National Institute on Drug Abuse Household Survey

The recently completed 1985 National Household Survey on Drug Use provides the national probability data on current use and correlates of use of smokeless tobacco by youth. It is the eighth in a series of national probability surveys conducted among household residents in the coterminous United States by the National Institute on Drug Abuse (NIDA). Data are collected on the use and adverse consequences that are associated with 11 drugs or drug classes. The 1985 survey oversampled for blacks and Hispanics and younger age groups. The total sample consists of approximately 8,000 face-to-face interviews. The data presented here are based on a preliminary analysis of 4,564 interviews. Provisional estimates are presented in tables 7 through 9.

Sixteen percent of males under the age of 21 reported using chewing tobacco or snuff within the last year, in contrast to 11 percent of older males (table 7). The decline in older age groups is seen more clearly when narrower age categories are used (table 8). An estimate of the prevalence of weekly use may be obtained by combining the use frequency

TABLE 9.—Frequency of Smokeless Tobacco Use in Past Year*

	Percentage of Users				
	Age Groups for Males				Males and Females
Past Year Use of Smokeless Tobacco	12-17	18-25	26-39	40+	Age 12 and Above
Most Days/Week	3	7	5	4	2
1 or 2 Days/Week	2	1	1	1	1
1 or More Days/Week	5	8	6	5	3
3-51 Days/Year	5	5	3	3	2
1-2 Days/Year	6	3	2	1	2
Not in Past Year	4	7	5	8	3
Have Tried	20	23	15	16	10
Never	80	77	85	84	90

* Preliminary estimates not adjusted for oversampling of blacks and Hispanics.

Source: National Institute on Drug Abuse, 1985 National Household Survey on Drug Abuse. Preliminary results presented at the NIH Consensus Development Conference on the Health Implications of Smokeless Tobacco Use, January 1986.

categories of "most days a week" and "1 or 2 days a week" (table 9). Use at least once a week peaks in the 18- to 25-year-old age groups at 8 percent. As in previous surveys, the use among females was consistently much lower than among males. Responses suggest slightly higher rates of use among women 40 years of age and older than among younger women (table 8) *(25)*.

Discussion of National Survey Data

Despite varying methodologies among the national surveys (table 2), sufficient commonalities permit meaningful comparisons. The 1970 and 1975 OSH surveys and the 1980 to 1985 Simmons Study of Media and Markets indicate that the use of snuff by adult males remained constant within a range of 3 to 4 percent. Use by adult females also remained constant at about 1 percent. During this same 15-year period, the population over the age of 18 increased 32 percent from 133.5 million to 175.8 million *(26)*. The production of all forms of smokeless tobacco increased 42 percent from 95.2 to 135.6 million pounds, and the production of fine-cut/moist snuff tripled. This may indicate the emergence of a new population of users.

The 1970 NHIS and the 1985 CPS both relied on the use of proxy respondents. Estimates of smokeless tobacco use are likely to be lower than the actual population prevalence because respondents may not always be aware of smokeless tobacco use by other members of the household. In fact, in 1970, the NHIS estimated that 1.4 percent of males used snuff and 3.8 percent used chewing tobacco. In the same year, the OSH Adult Survey, which did not use proxy respondents, provided corresponding estimates of 3 and 6 percent. Similarly, the CPS estimates that 1.9 percent of males used snuff in 1985, while the Simmons Study of Media and Markets estimates 3.2 percent.

However, comparisons between the 1970 NHIS and the 1985 CPS for the purpose of examining trends are appropriate. They suggest little change in the overall rate of adult male use of smokeless tobacco but indicate a marked change in the age distribution of users (table 4). In 1970, the use of smokeless tobacco was most common among older men; in 1985, the prevalence in the younger age groups had greatly increased.

Both the Simmons Study of Media and Markets and the NIDA survey show the highest rates of use among young adults ages 18 to 24. The Simmons National College Study indicates that male college students are as likely to use snuff as are other 18- to 24-year-olds. The Simmons data also show a slight elevation in prevalence among persons over the age of 65, which reflects the age distribution of traditional users of smokeless tobacco.

If the NIDA prevalence estimates are applied to current population figures *(26)*, there are at present over 12 million persons in the United States ages 12 and older who have used some form of smokeless tobacco in the past year. Three million are under the age of 21, and 1.7 million of these are males 12 to 17 years old. An estimated 6 million persons use smokeless tobacco at least weekly. Of these, 0.5 million are males ages 12 to 17; 1.3 million are males ages 18 to 25; and approximately 780,000 are females.

The 1980 to 1985 Simmons Study of Media and Markets estimated that 2 to 4 million persons over the age of 18 were users of snuff. Of these, 0.6 to 1.2 million were between the ages of 18 and 24.

Table 10 summarizes data on the prevalence of smokeless tobacco use by region from three national surveys conducted in 1985. Among these adult samples, use was highest in the South and lowest in the Northeast, with the West and North Central/Midwest falling in between.

These surveys provide self-report data only; no direct validation attempts were made. Because no strong social sanctions regarding smokeless tobacco use exist for adults, systematic misrepresentation by them is unlikely. However, under the conditions of a personal interview, as used in the NIDA study, adolescents would be more likely to underreport than overreport their use of smokeless tobacco. In addition, the preliminary estimates from the NIDA survey have not been adjusted for oversampling of blacks and Hispanics. In this sample, blacks and Hispanics reported less smokeless tobacco use than whites, and their overrepresentation would result in underestimates of national prevalence.

State and Local Survey Data

State and local surveys provide much of the information after 1980 on the use of smokeless tobacco. Since most of these surveys were conducted in schools, often motivated by apparent increases in students'

TABLE 10.—Prevalence of Smokeless Tobacco Use by Census Region, 1985

Prevalence Category	Percentage Reporting Use			
	Northeast	North Central	South	West
CPS				
Chewing Tobacco	1.6	3.7	7.0	3.9
Snuff	1.2	2.3	3.1	1.6
Simmons				
Snuff	1.5	1.3	2.9	1.3
NIDA* (Snuff and/or chewing tobacco)				
Weekly Use or More Often	1.0	2.0	5.0	4.0
Any Use in Past Year	4.0	6.0	8.0	9.0

* Preliminary estimates not adjusted for age and race.

Sources: Office on Smoking and Health, Current Population Survey, 1985 (unpublished). Simmons Market Research Bureau, Inc., Study of Media and Markets, 1980-1985. National Institute on Drug Abuse, 1985 Household Survey on Drug Abuse. Preliminary results presented at the NIH Consensus Development Conference on the Health Implications of Smokeless Tobacco Use, January 1986.

use of smokeless tobacco products, there may be a selection bias. However, the large and growing number of reports and the wide geographic coverage support the conclusion that smokeless tobacco use is not a localized phenomenon. Indeed, the consistency of such data suggests that smokeless tobacco has become a product that is used by large numbers of teenage and young adult males.

Adult Use

Several reports provide a tentative profile of local usage patterns of smokeless tobacco among adults. In 1979, tobacco use information was collected from 4,282 men between the ages of 21 and 84 in 10 geographic areas as part of the National Bladder Cancer Study, a population-based case control study *(27)*. The overall prevalence for having "ever used snuff for 6 months or more" among the control subjects (randomly selected from the general population) was 5 percent; for chewing tobacco, the corresponding figure was 12 percent. A breakdown by age indicated much more use of smokeless products by older men than younger men (table 11).

Glover and his colleagues conducted a random sample telephone survey of 280 persons in Pitt County, North Carolina *(28)*. A user was defined as a person who answered "yes" to the question, "Do you dip or chew tobacco?" Forty percent of males and 9 percent of females answered positively. High rates of use are probably not a new phenomenon since there is a tradition of smokeless tobacco use among both sexes in this area, and tobacco is a major agricultural product.

TABLE 11.—Prevalence of Snuff and Chewing Tobacco Use by Adult Males in 10 Geographic Areas

Sample	n	Percentage Reporting Ever Used	
		Snuff	Chewing Tobacco
All Men	4,282	5	12
Age			
21-44	240	0	2
45-64	1,653	3	6
65-84	2,389	7	16
Area of Residence			
Atlanta	186	8	23
Connecticut	654	4	12
Detroit	355	8	20
Iowa	552	12	14
New Jersey	1,288	2	10
New Mexico	129	7	20
New Orleans	115	1	6
San Francisco	542	2	8
Seattle	255	10	6
Utah	206	5	7
Race			
White	3,892	5	11
Nonwhite	390	5	18

Source: National Bladder Cancer Study. Hartge, P., Hoover, R., and Kantor, A. Bladder cancer risk and pipes, cigars, and smokeless tobacco. *Cancer*, 55: 901-906, 1985. Research supported by the National Cancer Institute, the Food and Drug Administration, and the Environmental Protection Agency.

Gritz, Ksir, and McCarthy surveyed a sample of 214 students at the University of Wyoming *(29)*. In their sample, 27.1 percent of males and 4.1 percent of females reported "current use," with the criterion for "current use" unspecified. The vast majority of users (84 percent) used moist snuff.

Glover and his colleagues reported a survey of 5,894 students in physical education classes at 72 colleges and universities from 8 States (Oregon, Arizona, Colorado, Oklahoma, Minnesota, Ohio, South Carolina, and Connecticut) *(30)*. Twenty-two percent of the males who were surveyed reported using smokeless tobacco compared to 2 percent of the females. Combined rates of use for both sexes ranged from 15 percent in Oklahoma to 8 percent in Connecticut. The majority of the users reported using less than one can or pouch per week.

Adolescent Use

Studies of school-age youth conducted since 1980 are summarized in table 12 *(31-45)*. Five different criteria for classifying use have been selected for data display: daily use, weekly use, monthly use, current use (no frequency specified), and ever used.

Recent regional data on the use of smokeless tobacco have been collected by a number of National Cancer Institute grantees in the course

TABLE 12.—Prevalence of Use of Smokeless Tobacco Among Youth by Gender and Grade: Regional and State-Level Surveys Reported Since 1980*

Location (reference)	Grade(s)	Males	Females	Total	n
Daily Use					
Arkansas (31)	10-12	26.0	—	—	179
Arkansas (32)	10-12	—	—	15.0	901
Nebraska (33)	7-12	2.5	0.0	—	2,612
Ohio (34)	4-12				
Chewing Tobacco		11.4	0.2	—	1,004
Snuff		19.7	0.4	—	1,004
Oregon (35)	7	8.8	0.7	—	443
	9	18.5	0.0	—	249
	10	23.1	2.4	—	130
Oregon (36)	7	4.6	—	—	710
	8	5.8	—	—	139
	9	9.7	—	—	432
	10	10.6	—	—	255
Wisconsin (37)	7	3.0	0.0	—	—
	8	6.0	0.0	—	—
	9	3.0	0.0	—	—
	10	8.0	0.0	—	—
	11	11.0	0.0	—	—
	12	15.0	0.0	—	—
	Total	—	—	—	—
Weekly Use (Or more often)					
Nebraska (33)	7-12	4.8	0.0	—	2,616
Wisconsin (37)	7	12.0	—	—	—
	8	18.0	—	—	—
	9	15.0	—	—	—
	10	24.0	—	—	—
	11	25.0	—	—	—
	12	37.0	—	—	—
	Total	—	1.0	—	25,000
Monthly Use (Or more often)					
Arizona (38)	8-12	18.4	—	—	1,080
Midwestern States (39)	10-12	33.0	0.0	—	323
Nebraska (33)	7-12	7.1	0.0	—	2,616
Current Use (Frequency not specified)					
Arkansas (31)	10-12	31.8	2.2	—	179
Arkansas (32)	10	—	—	13.8	326
	11	—	—	20.6	330
	12	—	—	23.7	245
	Total	36.7	2.2	—	901

TABLE 12.—Continued

Location (reference)	Grade(s)	Males	Females	Total	n
Current Use (Cont.)					
Colorado (40)	10-12	21.6	0.6	—	1,119
Colorado (41)	10-12	26.0	0.0	—	445
Louisiana (42)†					
1976-1977					
Chewing Tobacco	8-9	11.0	—	—	—
	10-11	17.0	—	—	—
	12-13	25.0	—	—	—
	14-15	24.0	—	—	—
	16-17	15.0	—	—	—
Snuff	8-9	4.0	—	—	—
	10-11	7.0	—	—	—
	12-13	5.0	—	—	—
	14-15	11.0	—	—	—
	16-17	5.0	—	—	—
	Total	—	—	—	2,880
1981-1982					
Chewing Tobacco	8-9	24.0	—	—	—
	10-11	32.0	—	—	—
	12-13	39.0	—	—	—
	14-15	43.0	—	—	—
	16-17	15.0	—	—	—
	Total	—	—	—	1,981
Snuff	8-9	21.0	—	—	—
	10-11	26.0	—	—	—
	12-13	32.0	—	—	—
	14-15	30.0	—	—	—
	16-17	14.0	—	—	—
	Total	—	—	—	1,981
Pennsylvania (43)	7-12	30.0	0.0	—	538
Texas (44)	7-12	19.0	0.0	—	5,392
Wyoming (29)	7-9	24.5	1.2	—	2,408
Ever Used					
Arkansas (45)	K	—	—	21.4	112
Ohio (34)					
Chewing Tobacco	4-12	58.0	12.0	—	—
	Total	—	—	—	1,007
Snuff	4-12	64.0	24.0	—	—
	Total	—	—	—	1,007
Oregon (35)	7	63.4	19.9	—	445
	9	72.7	16.4	—	249
	10	76.7	23.8	—	133
Wisconsin (37)	7	32.0	—	—	—
	8	45.0	—	—	—
	9	47.0	—	—	—
	10	50.0	—	—	—
	11	47.0	—	—	—
	12	48.0	—	—	—
	Total	—	—	11.0	25,000

* Unless otherwise indicated, figures represent the usage of chewing tobacco and/or snuff. Multiple entries have been made for studies that provide for more than one classification criterion.

† Age listed rather than grade.

of their ongoing research on tobacco use by youth *(46)*. Through collaboration, these investigators have achieved more standardization in data collection than in previous studies, which makes comparisons among the different locales more meaningful. Although there were some differences in methodology, all of the studies addressed one or both of the following research questions:

1. What percentages of males and females have ever used smokeless tobacco?
2. What percentages of males and females have used smokeless tobacco in the last 7 days?

Adolescent males may be subject to pressures that simultaneously discourage and encourage smokeless tobacco use. Underreporting of use may result from the presence of teachers and the setting in which the survey is administered. Overreporting may result from peer pressure to be seen as a smokeless tobacco user. Accurate reporting may be facilitated by collecting breath or saliva samples when surveys are completed. Respondents who believe that their self-reports can be objectively verified via biochemical testing tend to provide more accurate responses *(47-49)*. Biochemical validation was used in 14 of the 17 subsamples reported in table 13.

Most studies do not distinguish between snuff and chewing tobacco. In reports where the two have been separated, both substances were found to be in use *(34,42,43)*.

Rates of smokeless tobacco use were consistently higher among males than females. This difference is especially marked when more precise classifications for regular use are employed. While substantial numbers of adolescent females report having tried smokeless tobacco at least once, very few use it on a regular basis *(33-35,37,39,46)*.

The use of smokeless tobacco by youth was generally higher in rural than urban areas, in small communities, and in areas where there is a tradition of smokeless tobacco use *(34,37,46)*. However, high rates of use have also been reported in large metropolitan areas as well *(37,40,46)*.

Table 14 summarizes data on smokeless tobacco use by ethnic groups collected by investigators using standardized questions *(46)*. To date, little information has been available on smokeless tobacco use by nonwhites, and some early research suggested that minority youth were not taking up the practice *(42)*. In these studies, however, Hispanic youth showed rates of smokeless tobacco use comparable to whites, and Native American rates were consistently higher. In most locales, use was less common among Asians and blacks. Nationally, black college students are less likely to use snuff than are white college students (table 6). Prevalence estimates for smokeless tobacco use by black adults, however, have equaled or exceeded those of whites (tables 5 and 11).

The likelihood of using smokeless tobacco appears to increase with age as well as over time *(32-35,37,42,46)*. Only one study has collected

TABLE 13.—Prevalence of Use of Smokeless Tobacco Among Youth by Gender and Grade: Local Surveys Using Standardized Questions

		Males		Females	
Sample	**Grade**	**Percentage**	**n**	**Percentage**	**n**
Used in Last 7 Days					
California Suburban/Rural	6	4.7	(469)	0.7	(407)
	7	14.8	(574)	1.4	(557)
	8	9.2	(487)	1.6	(499)
Minnesota Suburban/Urban	9	18.1	(2,015)	2.4	(2,146)
Montana Urban	4	9.4	(477)	2.0	(403)
	5	11.9	(429)	1.5	(392)
	6	13.9	(446)	3.2	(402)
New York Urban	4	3.9	(306)	0.3	(298)
	5	2.9	(272)	0.4	(275)
	6	10.7	(252)	0.4	(243)
New York New York City	6	1.1	(1,488)	0.9	(1,494)
New York Suburban	7	3.0	(2,016)	0.0	(1,811)
Oregon Suburban/Rural	6	6.0	(602)	0.9	(542)
	7	9.1	(627)	0.8	(618)
	8	13.6	(663)	1.0	(608)
	9	17.3	(572)	0.5	(567)
	10	22.2	(514)	2.3	(471)
	11	22.7	(440)	0.5	(431)
Oregon Suburban/Urban	6	1.9	(571)	0.4	(525)
	7	4.6	(570)	1.4	(575)
	8	6.8	(514)	0.8	(533)
	9	14.8	(588)	1.2	(575)
Southeastern United States 10 SMSA's	6	9.8	(305)	1.3	(228)
	7	12.1	(346)	0.6	(325)
	8	10.4	(279)	1.6	(313)
Vermont Rural	5	9.3	(288)	0.3	(317)
	6	14.9	(328)	1.0	(289)
Vermont Urban	4	2.8	(216)	0.0	(199)
	5	4.8	(207)	1.0	(201)
	6	5.4	(204)	0.0	(193)
Washington Rural	4	4.4	(45)	0.0	(47)
	5	6.4	(141)	1.3	(156)
	6	8.8	(968)	2.1	(964)
	7	13.1	(521)	4.1	(514)
	8	14.8	(316)	5.2	(325)
Washington Rural	10	23.7	(215)	0.4	(233)

TABLE 13.—Continued

Sample	Grade	Males		Females	
		Percentage	n	Percentage	n
Ever Used					
California Suburban/Rural	6	32.6	(473)	7.8	(411)
	7	56.2	(578)	19.6	(567)
	8	56.7	(492)	20.0	(504)
California Los Angeles SHARP	7	24.9	(273)	6.7	(310)
California Los Angeles SMART	7	25.3	(479)	7.7	(480)
	8	31.9	(429)	8.1	(418)
California Los Angeles TVSP	8	32.0	(1,240)	6.9	(1,474)
Minnesota Suburban/Urban	9	62.1	(2,001)	22.9	(2,133)
Montana Urban	4	41.0	(480)	17.5	(401)
	5	56.9	(431)	19.3	(394)
	6	68.2	(443)	24.6	(402)
New York Urban	4	23.1	(307)	3.4	(298)
	5	33.5	(272)	5.1	(275)
	6	47.8	(255)	7.0	(243)
New York New York City	6	6.7	(1,488)	3.0	(1,494)
New York Suburban	7	25.3	(2,016)	4.1	(1,811)
Oregon Suburban/Rural	6	48.3	(607)	16.2	(551)
	7	57.9	(639)	19.8	(630)
	8	64.5	(677)	23.8	(617)
	9	70.4	(577)	26.7	(576)
	10	74.7	(522)	31.1	(485)
	11	77.5	(445)	34.2	(436)
Oregon Suburban/Urban	6	32.4	(568)	8.7	(528)
	7	44.9	(568)	16.8	(572)
	8	54.1	(512)	17.2	(535)
	9	61.3	(589)	24.7	(575)
Southeastern United States 10 SMSA's	6	47.6	(309)	11.4	(229)
	7	49.0	(353)	13.5	(325)
	8	51.4	(280)	15.6	(314)
Vermont Rural	5	38.8	(289)	8.2	(317)
	6	54.8	(332)	7.2	(290)
Vermont Urban	4	17.4	(213)	3.0	(200)
	5	26.2	(207)	5.5	(201)
	6	39.8	(206)	3.1	(193)
Washington Rural	4	15.6	(45)	0.0	(47)
	5	27.0	(141)	7.7	(156)
	6	49.0	(968)	13.0	(964)
	7	52.0	(521)	16.0	(514)
	8	58.9	(316)	20.1	(325)
Washington Rural	10	73.5	(215)	30.9	(233)
Waterloo, Canada Suburban/Rural	11	26.0	(281)	5.5	(444)

TABLE 14.—Mean Frequency of Smokeless Tobacco Use During Last 7 Days by Ethnicity of Male Respondents

Sample	Ethnicity	Prevalence	
		n	%
California	Asian	192	3.7
Suburban/Rural	Black	118	6.1
Grades 6-8	Hispanic	188	11.2
	White	1,046	11.4
Minnesota	Asian	36	13.9
Suburban/Urban	Black	201	4.0
Murray	Hispanic	24	45.8
	Native American	38	18.4
	White	1,602	19.6
New York	Asian	119	2.5
New York City	Black	205	0.5
Grade 6	Hispanic	510	1.0
	White	501	1.2
New York	Asian	23	4.3
Suburban	Black	47	2.1
Grade 7	Hispanic	39	2.6
	Native American	26	3.8
	White	1,796	3.3
Oregon	Asian	38	5.3
Suburban/Rural	Black	33	15.2
Grades 6-11	Hispanic	61	16.4
	Native American	120	23.3
	White	3,162	14.2
Oregon	Asian	71	2.8
Suburban	Black	231	3.9
Grades 6-9	Hispanic	26	0.0
	Native American	48	12.5
	White	1,847	7.6
Southeastern	Black	258	3.9
United States	White	652	14.0
10 SMSA's			
Washington	Asian	148	6.1
Rural	Black	119	1.7
Grades 4-8	Hispanic	111	9.0
	Native American	179	30.7
	White	1,434	9.4

both cross-sectional and longitudinal data. Hunter and her colleagues assessed tobacco use by children in Bogalusa, Louisiana, in 1976-77 and again in 1981-82 *(42)*. The use of both snuff and chewing tobacco increased over time within age categories, within age cohorts, and across age categories (table 12). A decrease in use was observed in the oldest age category, 16-17 years old, but has not been seen in other locales (tables 12 and 13). The decrease may reflect age-related changes in normative behavior particular to that area or a cohort effect.

Peer and family members are found consistently to be important influences on smokeless tobacco use by children and adolescents. Young users of smokeless tobacco have more friends who also use smokeless tobacco *(34-36,39,45)* and may themselves identify friends' encouragement as a reason for use *(35,44)*. Users of smokeless tobacco are also more likely to have family members who themselves use smokeless tobacco *(34,36,45)* and encounter less parental disapproval of the practice *(31,34)*.

In a special National Program Inspection study prepared by the Office of the Inspector General of the Department of Health and Human Services, young current and former users of smokeless tobacco were interviewed in depth *(50)*. Two hundred and ninety students in junior and senior high schools from 16 States volunteered to participate. All had used smokeless tobacco on a weekly or daily basis. While this study was not designed to provide prevalence estimates, it provides useful information about the attitudes and practices of some adolescent smokeless tobacco users.

Over 90 percent of these respondents used snuff exclusively, and over 55 percent indicated that they would have strong cravings if they tried to quit. On the average, this group reported first trying snuff at age 10 and beginning regular use by age 12. Fifty percent cited pressure from friends as their primary reason for initiating use, but continued use was most often attributed to enjoyment of taste (64 percent) and habit strength ("being hooked," 37 percent). Over 85 percent thought that dipping and chewing can be harmful to health, but less than 55 percent considered regular use to present a moderate or severe risk.

CONCLUSIONS

1. Recent national data indicate that over 12 million persons used some form of smokeless tobacco (chewing tobacco and snuff) in 1985 and that approximately 6 million used smokeless tobacco weekly or more often. Use is increasing, particularly among young males.

2. The highest rates of use are seen among teenage and young adult males. A recent national survey indicates that 16 percent of males between 12 and 25 years of age have used some form of

smokeless tobacco within the past year and that from one-third to one-half of these used smokeless tobacco at least once a week. Use by females of all ages is consistently less than that of males; about 2 percent have used smokeless tobacco in the last year.

3. State and local studies corroborate the national survey findings. The prevalence of smokeless tobacco use by youth and young adults varies widely by region, but use is not limited to a single region. In several parts of the country, as many as 25 to 35 percent of adolescent males have indicated current use of smokeless tobacco.

RESEARCH NEEDS

More systematic and detailed national and local surveys on smokeless tobacco should be conducted.* National probability sample surveys need to be supplemented with surveys of suspected "hot spots" to detect the extent of high-risk areas in the country and the prevalence of use in these areas.

Standardized methods are essential to facilitate appropriate comparisons among data. The current state of assessment is similar to the early days of research on cigarette smoking before standardized formats for assessment of prevalence and quantification of dosage became available. Accurate and reproducible dosage measurement for smokeless tobacco products is needed. Standardization may prove more difficult than for cigarette smoking because of the multiplicity of product forms.

Specific items that require standardization include the following:

- Collection of data separately for snuff and chewing tobacco.
- Definition of user classified according to the frequency of use. To date, little attention has been given to finer distinctions of use, including quantity used, the appropriate unit of measurement, and time that the product is allowed to remain in the mouth.
- Description of use. Data need to be gathered on patterns of use as well as the relationship of use to cigarette smoking.
- Reporting of age of initiation and duration of use.
- Definition of quit attempts and a quitter.
- Natural history of smokeless tobacco use and its relationship to other substance use, including other forms of tobacco, particularly cigarettes.
- Surveys of adequate sizes to permit stratification of the samples by relevant variables such as gender, age, ethnicity, socioeconomic status, cigarette smoking status, and various behavioral factors such as attitudes and knowledge, peer pressure, and academic status.

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* The 1986 OSH Adult Use of Tobacco Survey will address many of the items listed below.

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Chapter 2.

CARCINOGENESIS ASSOCIATED WITH SMOKELESS TOBACCO USE

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INTRODUCTION

This chapter presents the results of a systematic review of the world's medical literature describing experimental and human evidence pertinent to the evaluation of smokeless tobacco as a potential cause of cancer. Five categories of research relevant to assessing the role of smokeless tobacco in cancer causation were defined:

1. Epidemiologic studies and case reports of oral cancer in relation to smokeless tobacco use.
2. Epidemiologic studies of other cancers in relation to smokeless tobacco use.
3. Chemical constituents of smokeless tobacco.
4. Metabolism of constituents of smokeless tobacco.
5. Experimental studies involving exposing laboratory animals to smokeless tobacco or its constituents.

Consensus summaries of the literature in each of these categories were prepared and form the basis of this report. In addition, recommendations for future rsearch to clarify suggestive findings or fill gaps in knowledge are made.

EPIDEMIOLOGIC STUDIES AND CASE REPORTS OF ORAL CANCER IN RELATION TO SMOKELESS TOBACCO USE

Because smokeless tobacco products used in different regions of the world vary considerably in composition and usage patterns, this section will consider North American and European data separately from Asian data. Citations to the literature from India and other Asian countries where quids containing tobacco and other ingredients are commonly used orally focus on articles that attempt to distinguish tobacco from other ingredients in the quids as possible determinants of cancer risk.

Data From North America and Europe

Although about a dozen informative epidemiologic studies of smokeless tobacco use and oral cancer in North America or Europe have been reported, only a few were specifically designed to examine this relation. There are two major reasons for the relative paucity of studies. Apart from the recent increased prevalence in use of smokeless tobacco, the habit has not been widely practiced in America during this century, except in localized areas such as parts of the rural South *(1,2)*. Furthermore, cancer of the mouth is uncommon in the Western Hemisphere, exacerbating the difficulty of conducting epidemiologic investigations, particularly cohort studies, into the relation between smokeless tobacco and oral cancer. The age-adjusted incidence rate for cancers of the buccal cavity and pharynx in the United States is approximately 11 cases per 100,000 population per year, with these tumors accounting for about 3 percent of all cancer deaths *(3)*. Nevertheless, sufficient information is available to evaluate whether the use of smokeless tobacco increases the risk of oral cancer.

Case Studies

In their review of 566 oral cancer patients treated in two hospitals in Nashville, Rosenfeld and Callaway *(4,5)* noted that the proportion of women (61 percent) with buccal and gingival carcinoma was higher than the proportion of men (36 percent). Approximately 90 percent of women with buccal and gingival carcinoma used snuff for 30 to 60 years; in contrast, 22 percent of women with cancers in other oral cavity subsites used snuff. Many of these women began practicing "snuff dipping," namely, the placement of tobacco snuff in the gingivobuccal sulcus, between the ages of 10 to 20 years. These reports are typical of numerous and sometimes large series of cases from the South, which reported that high percentages of patients with gingivobuccal cancers were snuff dippers or tobacco chewers *(6-13)*. The articles describing these case series generally did not use comparison (control) groups, but the authors consistently commented on an apparently high prevalence of the use of snuff by the cancer patients. Clinicians also noted that the usual male predominance for epidermoid carcinomas of the oral cavity diminished or disappeared for the subgroup of gingivobuccal carcinomas occurring in geographic areas where there was relatively common use of snuff and chewing tobacco.

Ahblom reported in the 1930's on a possible association between smokeless tobacco and cancer in Sweden *(14)*. Among male patients with cancers of various sites seen at the Radiumhemmet (Stockholm), the use of snuff or chewing tobacco was reported in 70 percent with buccal, gingival, and "mandibular" cancers as compared to 26 to 37 percent with cancers in other oral subsites, the larynx, pharynx, and esophagus. Axéll et al. reviewed medical records of male patients with squamous cell carcinoma in the oral cavity diagnosed between 1962 and 1971 and recorded in the Register of the Swedish Board of Health and Welfare *(15)*. The authors were only able to determine a history for the pattern of use of snuff in 25 percent of eligible patients but commented that two-thirds of patients who were verified snuff users had oral cancers in regions where the snuff was generally placed. Reports of a single or a few cases, usually among male tobacco chewers, in the Northern United States and Canada also described buccal carcinomas that were often located precisely in the area where the tobacco was retained in the mouth *(16-19)*.

In the early 1940's, Friedell and Rosenthal associated the use of snuff or chewing tobacco with an exophytic, verrucous type of squamous carcinoma of the oral cavity *(16)*. Ackerman described in detail the morphologic and clinical features of verrucous carcinoma of the oral cavity *(20)*. Where the lesions originated in the buccal mucosa, a history of chronic use of chewing tobacco was elicited in 60 percent of the patients. The morphologic description was that of a well-differentiated, locally invasive, papillary squamous carcinoma, often in association with leukoplakia. In more than half of these patients, there was poor oral hygiene and carious and missing teeth.

In summary, clinical and pathological reports published during the past four decades in the United States and elsewhere have commented on the use of smokeless tobacco by oral cancer patients and have described the entity known as snuff-dipper's carcinoma *(4,7,11)*, providing the basis for the hypothesis that the prolonged use of snuff or chewing tobacco is associated with an increased risk of low-grade, verrucal or squamous cell carcinoma of the buccal mucosa and gingivobuccal sulcus.

Case Control Studies

Most of the epidemiologic evidence comes from several case-control studies of oral cancer. The low prevalence of smokeless tobacco use in most North American populations contributes to a low statistical efficiency in most of these studies. Good information has been obtained, however, from studies that were either very large, conducted in an area of high prevalence of smokeless tobacco use, or analyzed according to site within the oral cavity (since the tissue affected by snuff use appears to be highly localized). One study, by Winn et al., with these characteristics consequently provides the most informative body of data on the carcinogenicity of smokeless tobacco in North America *(21)*.

The major concern for validity in the epidemiologic studies of smokeless tobacco and oral cancer is uncontrolled confounding. A small number of subjects in crucial categories prevented efficient adjustment for confounding by stratification in many of these studies. Many of the studies were conducted before the advent of sophisticated epidemiologic analyses and make no attempt to control confounding. The two primary confounding factors of concern are alcohol consumption and smoking *(22)*. Alcohol consumption is a strong risk factor for oral cancer. It is not clear on a priori grounds, however, to what extent alcohol consumption would be correlated with smokeless tobacco use. The relation between smoking, also a strong risk factor for oral cancer *(2)*, and smokeless tobacco use may be complex. Users of smokeless tobacco may be more likely to have been smokers at some time. On the other hand, heavy users of smokeless tobacco typically cannot be heavy users of cigarettes, so that smoking is presumably negatively correlated with smokeless tobacco use. Failure to control confounding by smoking would therefore lead to underestimates of the effect of smokeless tobacco.

TABLE 1.—Smokeless Tobacco and Mouth Cancer, Case-Control Data From Moore et al. *(23,24)*

Smokeless Tobacco	Mouth Cancer Cases	Controls
Users	26	12
Nonusers	14	26
Totals	40	38
Crude RR = 4.0	95%-Confidence Interval:	1.6—10

Chronologically, the first case-control study of smokeless tobacco was conducted by Moore et al. in Minnesota (23,24). Patients at the University of Minnesota Tumor Clinic with a diagnosis of cancer of the mouth were interviewed about tobacco use as part of a general interview procedure for clinic patients. Surgical outpatients who received the same interviews served as controls. From the data that were reported by these authors, one can calculate a crude relative risk estimate for mouth cancer among smokeless tobacco users of 4.0 with a 95-percent confidence interval of 1.6-10 (table 1). An oddity was an apparent lack of effect for other forms of tobacco use. A partial explanation might be negative confounding between smokeless and smoked tobacco; indeed, 26 of the 40 cases of mouth cancer chewed tobacco. Still, the extent of disparity in crude effect estimates for smokeless tobacco (relative risk estimate 4.0) and smoked tobacco (all relative risk estimates <1.0) is surprising.

Wynder et al. reported on a case-control study of squamous cell cancers of the upper alimentary and respiratory tract that was conducted at Sweden's Radiumhemmet in 1952-55, including 33 tongue cancer patients, 14 lip cancer patients, 19 gingival cancer patients, and 8 patients with cancer of the buccal mucosa, among others (25). Controls were patients with cancers of the skin, head, and neck other than squamous cell carcinoma, stomach cancer, lymphoma, salivary-gland tumors, leukemia, sarcoma, cancers of the colon and rectum, and cancers of the female genital tract. A variety of risk factors was examined, including the use of chewing tobacco. The authors state that the data suggested that an increased risk is associated with the duration of chewing tobacco for cancers of the gingiva and oral cavity but not for cancers of the tongue, lip, hypopharynx, esophagus, or larynx, but the data as presented do not permit an estimation of risk. In addition, data were not adjusted for other potential confounders, including cigarette smoking. Wynder and colleagues also reported in 1957 data from a similar hospital-based case-control study of mouth cancer conducted in New York *(26)*. Tobacco chewing was found to be more common among men with oral cavity cancers than among controls; but it was noted that almost all of these patients also drank alcoholic beverages and smoked, and no further analyses were attempted.

TABLE 2.—Smokeless Tobacco and Mouth Cancer, Case-Control Data From Peacock et al. (27)

	Age					
	40-49		50-59		60-69	
Smokeless Tobacco	Case	Controls	Case	Controls	Case	Controls
User	0	16	7	13	18	20
Nonuser	5	14	6	16	9	37
Total	5	60	13	29	27	57
	RR = 0		RR = 1.4		RR = 3.7	
	RRMH = 2.0		95%-Confidence Interval: 1.0—4.2			

Peacock et al. studied 56 cases of mouth cancer, including malignancies of the buccal mucosa, alveolar ridge, and floor of the mouth, and compared their tobacco histories with those of two control groups: 146 hospitalized controls with diagnoses other than cancer and 217 outpatients *(27)*. Age-specific results using the hospitalized controls are summarized in table 2. The overall relative risk was estimated to be 2.0 (95-percent confidence interval 1.0-4.2); the relative risk seemed to increase with age with an estimate of 3.7 for the 60 to 69 age group. The data were not reported in sufficient detail to control for confounding by smoking, which presumably led to underestimates of the relative risk. There was also insufficient detail reported to evaluate the relation between the risk of mouth cancer and the amount or duration of smokeless tobacco use.

In Atlanta, patients with oral, pharynx, and larynx cancer were compared to three control groups having other mouth diseases, other cancers, or no cancer *(28)*. Among urban women, 40 percent of the cases used snuff compared to 3 percent or less of the controls (table 3). Among rural women, 75 percent dipped snuff compared to 20 percent or less among controls. Cigarette smoking was common in urban women and not specifically controlled for. Few rural female cases smoked cigarettes (7 percent) so confounding by smoking was minimal. The association between snuff dipping and oral, pharynx, and larynx cancer in women was generally evident in most age groups. Among the cases, the proportion of snuff dippers was highest among oral cancer patients: 53/72 were dippers compared to 2/18 pharynx and larynx cancer patients. Among men, insufficient information was provided to obtain precise epidemiologic estimates of the effect of chewing tobacco, although data from one of the bar charts presented indicate that urban cases were more likely to be users of smokeless tobacco than controls, that rural men with oral, pharynx, and larynx cancer or mouth disease were more likely to chew than controls, and that oral cancer patients were more likely to chew

TABLE 3.—Estimated Relative Risks Associated With Snuff Use for Cancers of the Oral Cavity, Pharynx, and Larynx, Case-Control Data From Vogler et al. *(28)*, Females Only

	Oral/ Pharynx/ Larynx	Other Mouth Disease	Other Cancer	No Cancer
Urban				
User	15	1	5	4
Nonuser	23	56	165	373
Crude Relative Risk Estimate	60.8	1.7	2.8	1.0*
Rural				
User	41	4	26	17
Nonuser	14	33	103	133
Crude Relative Risk Estimate	22.9	0.9	2.0	1.0*

* Reference category

TABLE 4.—Smokeless Tobacco and Head and Neck Cancer by Anatomic Site, Case-Control Data From Vincent and Marchetta *(29)*, Males Only

Smokeless Tobacco Use	Control	Larynx	Pharynx	Oral Cavity	All Head and Neck
User	5	2	3	9	14
Nonuser	95	21	30	24	75
Total	100	23	33	33	89
Relative Risk Estimate		1.8	1.9	7.1	3.5
95%-Confidence Interval		0.3—9.8	0.4—8.3	2.4—21	1.3—9.8

than the pharynx and larynx cancer cases. Among men, confounding by smoking could not be ruled out.

Vincent and Marchetta reported the results of a case-control study of head and neck cancer according to anatomic site. Table 4 summarizes the findings for males *(29)*. The oral cavity seems to be the anatomic site where the bulk of the effect is noted; only mild increases in risk were estimated for the larynx and pharynx, whereas users of smokeless tobacco were estimated to have a sevenfold greater risk for cancer of the oral cavity. These estimates are imprecise because of the small number of subjects and are uncontrolled for age and smoking.

TABLE 5.—Estimated Relative Risk for Cancer of the Head and Neck From Smokeless Tobacco Use by Anatomic Site, Third National Cancer Survey (31), Males Only

Anatomic Site	Relative Risk Estimate	
	Low Exposure	High Exposure
Gum-Mouth	5.6	3.9
Pharynx	0.6	—
Lip-Tongue	0.3	1.1
Larynx	2.0	1.7

Martinez reported on a case-control study in Puerto Rico of risk factors for cancers of the mouth, pharynx, and esophagus *(30)*. This population-based study included 400 cases of epidermoid carcinomas of those sites and 1,200 controls matched on age (±5 years) and sex to the cases. One control per case was drawn from the same hospital or clinic and two from the same community. There were 153 cases of mouth cancer (115 male and 38 female) and 68 cases of pharyngeal cancer (55 male and 13 female). The authors concluded that "Patients with cancer of the mouth did not often use chewing tobacco disproportionately . . ." However, calculation of the relative risks of mouth cancer that are associated with chewing tobacco based on comparing the use of chewing tobacco only with no tobacco use suggests a strong effect for oral and pharyngeal cancer in males (data from table 13 in the paper). The estimated relative risks were 11.9 (95-percent confidence interval 2.5-56.4) for oral cancer and 8.7 (95-percent confidence interval 1.4-54.5) for pharyngeal cancer among chewers. These numbers do not include the experience of the many study subjects whose use of tobacco was "mixed" (that is, those who used any combination of cigarette, cigar, and pipe smoking and chewing tobacco), and these calculations were based on unmatched data.

Further evidence for the site specificity arose from a case-control analysis of multiple cancers using data from the Third National Cancer Survey *(31)*. There were few female users of smokeless tobacco and scanty data by site within the head and neck region even for males; the findings do seem to indicate that the effect is greater for the site that is labeled gum-mouth as opposed to other head and neck sites (table 5).

Browne et al. conducted interviews with 75 oral cancer patients, or (usually) their next of kin, and 150 living sex-, neighborhood-, and occupation-matched controls in the West Midlands area of the United Kingdom where oral cancer mortality rates were high and tobacco chewing was common among miners *(32)*. Controls on average were born about 10 years earlier than the cases. The proportion of tobacco chewers was approximately the same among the 16 cases and 43 controls who were miners, although data on this variable were missing for one-fourth of the cases, and the authors apparently assumed that all cases with missing information were nonchewers. If the proportion of tobacco chewers among the cases with missing information was similar to those miners with known information, then the data would have shown a positive association between chewing tobacco and oral cancer. All of the miners with oral cancer who chewed tobacco also smoked pipes, further complicating interpretation of this study.

Additional evidence that a carcinogenic effect of smokeless tobacco may be greatest at the anatomic site of exposure came from Westbrook et al. who compared the medical records of 55 female patients with cancers of the alveolar ridge or buccal mucosa who were treated at the University of Arkansas with those of 55 randomly selected female hospital controls *(33)*. Fifty of the cases, but only one control, were snuff dippers, with the tumors among the cases typically appearing at the site where the snuff was usually placed. No reliable estimates of risk can be derived from this study because of the strong possibility that there was not comparable elicitation of exposure information for cases and controls.

Two large case-control studies were not reported in a way that enables a meaningful quantitative assessment of the effect of smokeless tobacco in chewers and dippers compared to tobacco abstainers *(34,35)*. The first study found that 10 percent, and the second 9 percent, of male oral cancer cases had ever chewed tobacco, while the corresponding figure for controls was 9 percent. These studies, like many of the others cited here, were not undertaken specifically to evaluate the carcinogenicity of smokeless tobacco. Although the data seem to indicate a weak relation, if any, between smokeless tobacco and cancer of the oral cavity, the findings are uncontrolled for age, race, geography, and smoking.

The recent case-control study of Winn et al. is by far the most informative study on the carcinogenicity of smokeless tobacco *(21)*. The case series comprised 255 women with oral and pharyngeal cancer who were living in 67 counties in a high-risk (for oral cancer) region of North Carolina. Two female controls were obtained for all but a few cases and were individually matched for age, race, source of ascertainment (hospital or death certificate), and county of residence. There was a fourfold increased risk of oral-pharyngeal cancer among nonsmoking white women who dipped snuff. The association could not be explained by smoking or alcoholic beverage consumption *(21)*, denture wearing or poor dentition *(36)*, diet *(37)*, or mouthwash use *(38)*. The data provided evidence for a strong relation between the duration of snuff use and risk for cancer, as well as a striking localization of the carcinogenicity to the gum and buccal mucosa (table 6). For long-term chronic users of snuff, there was nearly a fiftyfold increase in risk for cancers of the gum and buccal mucosa. Indeed, almost all of the patients with cheek and gum cancers had dipped snuff.

TABLE 6.—Estimated Relative Risk of Oro-Pharyngeal Cancer According to Duration of Snuff Use and Anatomic Site, Winn et al. *(21)*

Anatomic Site	Duration of Snuff Use (yr)	Relative Risk Estimate	95%-Confidence Interval
Gum and Buccal Mucosa	0	1.0	—
	1 – 24	13.8	1.9 – 98
	25 – 49	12.6	2.7 – 53
	≥ 50	48.0	9.1 – 250
Other Mouth and Pharynx	0	1.0	—
	1 – 24	1.7	0.4 – 7.2
	25 – 49	3.8	1.5 – 9.6
	≥ 50	1.3	0.5 – 3.2

Although some of the exposure information came from interviews with next of kin, when the analysis was restricted to interviews with study subjects, the association between snuff and oral cancer was even stronger *(39)*. Matched conditional logistic analysis yielded similar results *(39)*. Based on calculations of attributable risk, the authors estimated that 87 percent of these cancers were due to the patients' snuff-dipping habits. The authors also provided data that demonstrated the negative confounding by tobacco smoking in the population, raising the possibility of a serious validity problem with the other studies that did not control for smoking. If the negative correlation between the use of smokeless and smoked tobacco holds in other populations, estimates of the carcinogenic effect of smokeless tobacco in studies without the control of smoking may be underestimates. The quantitative information that was provided by the Winn et al. study led its authors to conclude that the long-standing use of smokeless tobacco by Southern women was the principal cause of the elevated mortality from oral cancer among women in the Southern United States.

Cohort Studies

Few cohort studies of smokeless tobacco have been undertaken because of the rarity of both the exposure (smokeless tobacco use) and the outcome (oral cancer) of most interest. Bjelke and Schuman *(40)* reported on cancer mortality in cohorts of 12,945 Norwegian men and 16,930 American men and found increases in the risk of death for cancers of the buccal cavity, pharynx, and esophagus (relative risk estimates ranged from 2.6 to 3.1 *(41)*; no further detail was given). They noted a negative association between smoking and chewing tobacco, confirming the pattern that was observed from the case-control research. In a 16-year followup of U.S. veterans, Winn et al. reported no deaths from oral or pharyngeal cancer among 951 smokeless tobacco users who did not use other forms of tobacco (about 0.5 deaths were expected) but a significant increase in both oral and pharyngeal cancers among smokeless tobacco users who were light smokers *(42)*. These data, as well as those from Bjelke and Schuman *(40)*, were reported only as abstracts in scientific journals or proceedings, with little or no detail as to the methods used, hindering interpretation of the results.

Smith and colleagues followed a group of about 1,500 patients with changes in the oral mucosa to evaluate the effects of smokeless tobacco use *(43,44)*. No oral cavity cancers were found in about 16,000 person-years of followup. Based on the results of other studies, two or three should have been detected over the study period. Smith gave little documentation of the methods that were employed for followup; however, 12 percent of the original group (201 subjects) were lost without any data on outcome, and there was apparently no effort to trace them. It seems likely that persons who died and persons who developed cancer, including some with tumors of the oral cavity, may have been lost to followup. In fact, no deaths among cohort members were reported, whereas perhaps as many as 100 or more would have been expected among such a cohort of middle-aged adults, making Smith's data uninterpretable.

Data From Asia

The highest rates of oral cancer among the more than 100 that are listed from population-based registries around the world that report standardized cancer incidence statistics are found in India *(45)*. In many areas of Asia, hospital statistics suggest that oral cancer is extremely common and often accounts for 25 or more percent of all cancers *(46-49)*, proportions that are far greater than in most areas of the United States where oral cancers typically comprise only 3 percent of all malignancies *(3)*. It has long been thought that the chewing of quids that contain tobacco and other substances is the cause of the increased risk of oral cancer in these areas *(50)*.

The smokeless tobacco products that are commonly used include tobacco with betel leaf, areca nut, and lime mixtures (often referred to as "pan"); Khaini (powdered tobacco and slaked lime paste); mishri (powdered, partially burnt black tobacco); nass (tobacco, ash, and cotton or sesame oil; lime is used in Iran and certain Soviet Republics); and various preparations that vary locally throughout the Southeast Asia region.

The inclusion of lime, areca nut, and other ingredients in many of the smokeless tobacco-containing quids hinders the evaluation of the contribution of tobacco per se to the increased risk of oral tumors. From five investigations, however, relative risks of oral cancer among chewers of betel quids with versus without tobacco can be calculated. Data from these case-control studies, which were conducted in Calcutta, Madras, Karachi, Bombay, and several parts of India and Sri Lanka *(47,51-55)*, reveal considerably higher risks of oral cancer for the

TABLE 7.—Relative Risk of Oral Cancer From Betel Quid With and Without Tobacco (With 95-Percent Confidence Limit)

Study Location (References)		Betel Quid With Tobacco	Betel Quid Without Tobacco	No Chewing Habit	Remarks
Calcutta, India *(50,54)*	Cases	138	46	135	Smokers not included in these data. Only buccal mucosa cancers considered.
	Controls	61	70	256	
	Relative risk estimates	4.3 (3.0-6.1)	1.2 (0.8-1.9)		
Madras, India *(51,54)*	Cases	219	33	25	Smokers not included in these data. Only buccal mucosa and tongue cancer cases included. Numbers reconstructed from percentages and totals.
	Controls	35	144	99	
	Relative risk estimates	25 (15-41)	0.91 (0.4-1.6)		
Karachi, Pakistan *(52,54)*	Cases	339	40	88	Smokers not included in these data.
	Controls	474	216	1,690	
	Relative risk estimates	14 (11-17)	3.6 (2.4-5.2)		
Bombay, India *(53)*	Cases	238	44	129	Separate analyses indicate that elevated risks of oral cancer associated with tobacco chewing are found among nonsmokers as well as smokers.
	Controls	513	152	1,340	
	Relative risk estimates	4.8 (3.9-6.0)	3.0 (2.1-4.3)		
India and Sri Lanka *(47)*	Cases	120	3	6	Smokers not included in these data. Only buccal mucosa cancer considered.
	Controls	63	8	47	
	Relative risk estimates	15 (7.0-32)	2.9 (0.6-14)		

use of tobacco-containing compared to nontobacco-containing quids (table 7). The findings thus suggest that the addition of tobacco contributes substantially to the elevated cancer risk among chewers, although other differences between those who use versus those who do not use tobacco-containing quids could influence the differences. Smoking, however, is not such a difference, since most of the investigations referred to in table 7 demonstrated high relative risks of oral cancer (with excesses among tobacco chewers often exceeding tenfold compared to nonquid users) among chewers who did not smoke, ruling out confounding by cigarette smoking. The studies also generally found that the large majority of oral cancer patients had been tobacco chewers and suggest that the habit of quid chewing accounts for most of the oral cancers in the diverse populations studied *(55,56)*.

Summary

Numerous case reports, especially in the South, have described oral cancers among smokeless tobacco users. The tumors often arose at anatomic locations where the tobacco was routinely placed. The number of epidemiologic investigations evaluating the relation between smokeless tobacco and oral cancer is not large, and several studies have methodologic limitations. The pattern of increased oral cancer risk among smokeless tobacco users, however, is generally consistent across studies, with evidence of an increasing risk with increasing duration of exposure, and with excess risks tending to be greatest for those anatomic sites where tobacco exposures are greatest. The best designed study was drawn from a female population in the Southern United States where exposure rates are high and potentially confounding variables could be taken into account. This study showed that chronic snuff users were at substantially increased risk of oral cancers and that nearly all tumors of the cheek and gum were due to snuff use. Evidence from parts of Asia, where the prevalence of smokeless tobacco use is high and oral cancer is the most common tumor, indicates a strong association between the chewing of quids and oral cancer. Users of quids that contain tobacco have much higher oral cancer rates than users of quids that do not, and the association is not confounded by cigarette smoking, raising the possibility that tobacco per se contributes to the elevated oral cancer risk in this part of the world. In summary, users of smokeless tobacco face a strongly increased risk of oral cancer, particularly for the tissues that come in contact with the tobacco.

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EPIDEMIOLOGIC STUDIES OF OTHER CANCERS IN RELATION TO SMOKELESS TOBACCO USE

The epidemiologic studies reported in the preceding section that show an association between the use of smokeless tobacco and oral cancers, particularly malignancies of the cheek and gum, indicate that the topical exposure of tissues to tobacco can cause cancers at the site of the exposure. In the United States, the tissues in direct prolonged contact with the tobacco are generally those of the oral cavity. Smokeless tobacco may occasionally come in contact with other tissues. One case has been reported of squamous cell carcinoma that developed in the ear of an individual in Minnesota who habitually placed snuff in his ear for 42 years at the site where the neoplasm developed *(1)*. Although but a single report, this highly unusual observation raises the possibility of a carcinogenic potential of smokeless tobacco at other anatomic sites when exposure is direct and prolonged.

Nasal Cancer

In some areas of the world snuff is inhaled, so that tissues of the nasal cavity come in contact with the tobacco powder. The earliest report that links any form of tobacco to cancer was published over two centuries ago when what were probably nasal cancers were described in several patients in England who were heavy inhalers of snuff *(2)*. There have been no systematic evaluations of snuff inhalation and nasal cancer in the United States, United Kingdom, or other European countries, most likely because both the sniffing habit and nasal cancer are uncommon. Sniffing snuff has been reported, however, to be a frequent habit among Bantu men, whose rates of nasal cancer have been reported to be high *(3)*. In case-control studies of nasal sinus cancer reported in 1955, 80 percent of patients with tumors of the maxillary antrum were prolonged and heavy snuff users, in contrast to about one-third of Bantu men with other cancers *(4,5)*. The snuff used by the Bantu is thought to contain aloe plant ash, trace elements such as nickel and chromium, and other ingredients in addition to tobacco *(6)*. Snuff use (presumably by inhalation) was reported not to account for the high rates of nasal adenocarcinoma among furniture makers in studies in England and Denmark, but evaluations of snuff itself as a risk factor were not undertaken *(7,8)*.

One case-control study of cancers of the nasal cavity and paranasal sinuses in the United States addressed the issue of smokeless tobacco *(9)*. A total of 193 cases were identified in four hospitals in Virginia and North Carolina over a 10-year period. No association between sinonasal cancers and chewing tobacco was found (relative risk 0.7, 95-percent confidence interval 0.4-1.5). However, a relative risk of 1.5 was observed for users of snuff (95-percent confidence interval 0.8-2.8). Risk was increased in snuff users for both adenocarcinomas (relative risk 3.1) and squamous cell carcinomas (relative risk 1.9) but not for other histologic types (relative risk 0.6) and was found for both sexes. The implications of the findings are not clear since the snuff used by the cases and controls was oral snuff not coming in contact with nasal tissues. Animal experiments, however, suggest that tumors distant to the site of exposure may result from exposure to constituents of snuff (see the section on animal studies).

An apparent excess of posterior nasal space tumors was reported among certain tribes in Kenya, and 6 or 12 cases interviewed were found to be chronic "liquid snuff" users *(10)*. Multiple subsites of the respiratory tract were considered, however, increasing the likelihood of a chance association. No increased risk of nasopharyngeal cancer associated with snuff use was noted in a case-control study in Singapore *(11)*.

Esophageal Cancer

Other tissues that come in contact with constituents of smokeless tobacco in more dilute concentrations include the linings of the esophagus, larynx (supraglotic portion), and stomach. The results of studies of

TABLE 1.—Relative Risks of Esophageal Cancer in Persons Exposed to Chewing Tobacco and Snuff: Summary of Four Case-Control Studies

First Author	Type of Exposure	Level of Exposure	Sex	Cases		Controls		Relative Risk*
				No.	% Exposed	No.	% Exposed	
Wynder *(12)*	Chewing	Any	M	150	20	150	10	2.3
		< 10 yrs.			14		4	3.9
		≥ 10 yrs.			6		6	1.2
Williams *(24)*	Chewing or Snuff	Level 1	M	38	5.2	1,788	5.4	0.9
		Level 2			0		0	—
Wynder *(13)*	Chewing	Any	M	183	10.9	2,560	9.0	1.2
	Snuff	Any	M		4.4		2.7	1.7
Martinez *(14)*	Chewing†	Any	M	120	2.5	360	3.6	1.2
			F	59	11.9	177	7.3	2.7

* Calculated from published report if not provided by author.

† Restricted to nonsmokers.

cancers of these three sites in relation to smokeless tobacco are inconclusive. The studies are generally of limited power to detect small increases in risk, and many did not control for relevant, potentially confounding variables. However, some studies of these three cancers do show an increase in risk in relation to the use of smokeless tobacco. As shown in table 1, elevated relative risks of esophageal cancer up to twofold or higher were found in two hospital-based case-control studies in the United States involving 150 and 183 cancer patients *(12,13)* and one in Puerto Rico (described in the previous section) with 179 cases *(14)*. One of the studies by Wynder and colleagues, however, found no evidence of an increase in risk with duration of exposure, and all chewers were also smokers *(12)*. The effect of smoking was not adjusted for in the other study *(13)*. Another case-control study involving 120 black male cases of esophageal cancer was conducted in Washington, D.C. (15). Few of the cases or controls had used either chewing tobacco or snuff, suggesting that it did not contribute to the high rates of esophageal cancer observed in the area. Finally, data from a prospective (cohort) study of U.S. veterans were analyzed to determine whether mortality rates of specific diseases were increased in users of smokeless tobacco *(16)*. In the absence of smoking, the standardized mortality ratio for esophageal cancer was found to be 228, but this value was based on only one death. In a cohort study of 12,945 Norwegian and 16,930 American men followed over 10 years, the risk of esophageal cancer was reported to be significantly increased among men who used chewing tobacco or snuff, after controlling for age, residence, and smoking habits *(17,18)*. Unfortunately, the results of both cohort studies have been published only as abstracts, so additional details are not available.

Some evidence that the chewing of quids may increase the risk of esophageal cancer arises from studies in Southeast Asia. In a series of 237 cases of esophageal cancers in Sri Lanka, interview information from 111 revealed that 90 (81 percent) habitually used betel containing tobacco leaf *(19)*. This percentage was considerably higher than the frequency of betel chewing in the general population (30 percent). Betel chewing was more common among women. Esophageal cancer also was more common among women, an unusual observation since this cancer occurs more frequently among men in almost all areas of the world that report standardized cancer statistics *(20)*. Since few women were reported to smoke or use alcohol, the possibility of an etiologic role of chewing is increased. However, the potential effects of tobacco as opposed to other ingredients in the quids cannot be distinguished. In a case-control investigation in Bombay involving interviews with 305 esophageal cancer patients and nearly 2,000 population controls of age, sex, and religions similar to all head and neck cancer cases, a 2.5-fold increased risk of esophageal malignancy was observed ($p < .01$) among nonsmokers who chewed pan, a mixture usually consisting of tobacco, betel, lime, and other ingredients *(21)*. The excess was higher, however, among those chewing quids without tobacco (relative risk 3.5) than with tobacco (relative risk 2.1). A more recent analysis *(22)* in Bombay based on 649 patients with esophageal cancer and 649 controls yielded similar qualitative findings, but the excess among users of pan without tobacco (relative risk 12.1) was accentuated compared to users of tobacco-containing chews (relative risk 2.8). On the other hand, in an earlier case-control investigation in southern India of several upper digestive tract tumors, including 93 esophageal cancers, increases in esophageal cancer risk were much greater among men who used betel with tobacco (calculated relative risk 11) than without tobacco (calculated relative risk 2) *(23)*.

The chewing of nass was not associated with esophageal cancer risk in a case-control study conducted in an area of Iran with among the world's highest rates for this cancer *(24)*. Of 638 identified cases of esophageal cancer, interviews were completed with 344 and with 2 neighborhood controls matched to each case. The relative risk associated with ever using nass was 0.9, with an upper limit of the 95-percent confidence interval of 1.5, suggesting that any major effect of nass on the origins of this cancer could be excluded.

Laryngeal Cancer

In a case-control analysis of the interview data from the Third National Cancer Survey (TNCS), Williams and Horm compared the prior use of smokeless tobacco products (in the aggregate) in persons with a variety of individual types of cancer (including laryngeal cancer) with the history of such use in persons with the remaining cancers thought not to be related to tobacco use *(25)*. Prior experience with smokeless tobacco was divided into two levels of exposure. The estimates of the relative risks were controlled for age, race, and smoking. Relative risks of laryngeal cancer in men of 2.0 and 1.7 were found among individuals with low and high levels, respectively, of exposure to chewing tobacco or snuff. These estimates were not significantly different from 1.0. They are based on 106 cases, 11 with relatively low exposure and 5 with higher exposure, and 2,102 controls of which 98 had low exposure and 71 had high exposure. Only 13 female laryngeal cases were available for analysis in this study, which was insufficient to provide any meaningful results.

A case-control study by Wynder and Stellman included 387 male cases of laryngeal cancer and 2,560 hospital controls *(13)*. The percentages that had previously used chewing tobacco and snuff were 11.9 and 3.9, respectively, for the cases, and 9.0 and 2.7, respectively, for the controls. Based on these findings, crude relative risks of 1.4 for chewing tobacco and 1.5 for snuff were obtained. Neither estimate differs significantly from 1.0. No control for smoking or alcohol was done, although the authors state that cigarette smoking in users and nonusers of chewing tobacco was similar.

Interviews with 560 laryngeal cancer patients and 2,000 controls from the general population of Bombay revealed significantly increased risks, compared to nonchewers, among chewers of betel without tobacco (relative risk 2.5) than with tobacco (relative risk 2.6) *(21)*. Laryngeal cancer was noted to comprise an unusually high proportion of all cancer diagnoses in a hospital series in eastern India where pan chewing is common, but no assessment of the role of tobacco was made *(26)*.

Stomach Cancer

Zacho et al. noted that, in Denmark, both gastric cancer and use of chewing tobacco and snuff are directly related to age, more common in men than women, more prevalent in rural than urban areas, and inversely related to socioeconomic status *(27)*. On the basis of these observations, they hypothesized that use of smokeless tobacco increases the risk of stomach cancer. Obviously, other differences among individuals within Denmark could also explain these findings.

Weinberg et al. conducted a case-control study of stomach cancer in a coal mining region of Pennsylvania *(28)*. Cases who had died of stomach cancer from 1978 through 1980 were compared with three control groups: persons who died of other cancers of the digestive system, persons who died of arterial sclerotic heart disease, and persons who lived in the same neighborhood as the case. All controls were matched to individual cases on age, sex, race, and location of residence. Data on the use of various forms of tobacco were obtained by interviewing next-of-kin or (for neighborhood controls) the subjects themselves. About 16 percent of all men in the study had used chewing tobacco. This percentage did not differ significantly among the cases and the three control groups. No women in this study had chewed tobacco. This study provides some evidence to suggest that chewing tobacco does not increase the risk of gastric cancer, although a small increase in risk could have been missed due to lack of statistical power.

The case-control analysis of the interview data from the TNCS found a relative risk of stomach cancer of 1.7 in men in the highest level of use of chewing tobacco and snuff, no increase in men in the lower use category, and no increase in women *(25)*. These results are based on 120 male cases, 12 of which were users, and 82 female cases, 2 of which were users. The power of this analysis to detect a true increase in risk is obviously low. The relative risk of 1.7 was not significantly greater than 1.0. In an abstract describing a cohort mortality study of U.S. veterans, the standardized mortality ratio for stomach cancer among nonsmoking users of smokeless tobacco was 151, but no study details were provided *(16)*.

Urinary Tract Cancer

Constituents of smokeless tobacco can enter the blood stream, and some are excreted in the urine. The kidney and bladder are thus potentially exposed to these agents but presumably in lower concentrations than are tissues of the upper aerodigestive tract. In a hospital-based case-control study in Seattle, Washington, patients who chewed tobacco were reported to be at nearly a fivefold increased risk of renal cancer compared to nontobacco users *(29)*. Only 6 percent of the 88 male cases were chewers. No association between the use of smokeless tobacco products and either renal cell or renal pelvis cancer was reported in a case-control study of these tumors in England *(30)*. Among 106 renal cell cancer case-control pairs in this study, 10 cases versus 11 controls had at some time used smokeless tobacco. Among 33 renal pelvis cancer-control pairs, 2 cases and 3 controls reported ever using smokeless tobacco products. In a large population-based study in Minnesota involving 495 cases and 697 controls, a nonsignificantly increased relative risk of renal cell cancer of 1.7 (95-percent confidence interval 0.5-6.0) was found among snuff users after adjusting for smoking *(31)*. There was a deficit in risk, however, associated with ever using chewing tobacco (relative risk 0.4, 95-percent confidence interval 0.1-2.6).

A review of eight epidemiologic investigations revealed no consistent evidence that the risk of bladder cancer is altered in users of smokeless tobacco products (table 2) *(13,25,32-39)*. The National Bladder Cancer Study is the largest of the investigations of bladder cancer considered in this review *(37)*. Cases for this study were selected through 10 population-

TABLE 2.—Estimates of Relative Risks of Bladder Cancer in Persons Who Have Ever Used Chewing Tobacco and Snuff

First Author (ref.)	Years Case Diagnosed	Sex	Relative Risks		
			Chewing Tobacco	Both	Snuff
Wynder *(32)*	1957-63	Male	1.4*		0.7*
Dunham et al. *(33)*	1958-64	Male	5.3*†	0.9*†	—
		Female	1.1*†	—	0.3*†
Cole et al. *(34)*	1966-68	Both	1.1*		1.0*
Williams and Horm *(25)*	1969-71	Male-level 1		1.61	
		level 2		1.15	
		Female-level 1		0	
		level 2		1.78	
Wynder and Stellman *(13)*	1974-75	Males	0.9		0.7
Howe et al. *(36)*	1974-76	Males	0.9		
Hartge et al. *(37)*	1977-78	Males	1.02		0.77†

* Estimated from published report.

† Based on analysis of nonsmokers only.

based cancer registries in the United States. Controls were a random sample of the same population from which the cases came. Information was obtained from interviews of 2,982 cases and 5,782 controls. Analyses of smokeless tobacco use were restricted to the 340 cases and 1,227 controls who claimed never to have smoked cigarettes. Of these, 11 percent of the cases and 10 percent of the controls had ever used chewing tobacco, and 3 percent of the cases and 4 percent of the controls had ever used snuff. The relative risks of bladder cancer in users of chewing tobacco and snuff were estimated to be 1.0 (0.7-1.5) and 0.8 (0.4-1.6), respectively.

Wynder et al. conducted a hospital-based study of 300 male bladder cancer cases *(32)*. Eleven percent of the 300 cases and 8 percent of the 300 hospital controls had ever used chewing tobacco; 2 percent of the cases and 3 percent of the controls had used snuff. The percentage of users was not significantly different in cases and controls, and no attempt was made to analyze the data further.

Dunham et al. interviewed 493 bladder cancer patients and 527 hospitalized controls in New Orleans *(33)*. Among nonsmokers, there was an increased relative risk associated with chewing tobacco use among males but a deficit in risk associated with snuff use among females, but the numbers of cases involved were small (four males and three females).

Cole et al. interviewed 470 cases from the Boston area and 500 population-based controls *(34)*. Forty-six of the cases had used chewing tobacco and three had used snuff. Based on the prior experience with smokeless tobacco in the controls (controlling for age and sex), 42.3 and 7.9 cases would have been expected to have used chewing tobacco and snuff, respectively. Some increase in the risk of bladder cancer was found in the TNCS survey, but none of the risks from this study are significantly different from 1.0 (table 1) *(25)*. In addition, no evidence of a dose response is seen.

In a second hospital-based case-control study *(13)* of similar design to the first *(32)*, Wynder and Stellman found that 8 percent and 1.9 percent of 586 cases had used chewing tobacco and snuff, respectively, compared to 9 percent and 2.7 percent of 2,560 controls who had used these two products. When analyses were restricted to nonsmokers in a continuation of this study, a significant excess risk of bladder cancer was associated with snuff use among women, but only 3 of 76 cases were users *(35)*.

A population-based case-control study was conducted in three Canadian provinces by Howe et al. *(36)*. Controls were matched to individual cases on neighborhood, age, and sex. The ratio of male pairs discordant for the use of chewing tobacco was 29/34, giving a relative risk of 0.9 (95-percent confidence interval, 0.5-1.6). This estimate was not altered by controlling for smoking. No female cases or controls gave a prior history of use of smokeless tobacco.

In Denmark, 165 male and 47 female patients with cancer of the urinary bladder from a hospital serving a specific geographic area were interviewed, as were geographically-matched controls *(38,39)*. The estimated relative risk associated with tobacco chewing was 2.0 (1.2-3.4) based on 39 exposed cases. In a logistic model containing variables for tobacco chewing, smoking, and other major correlates of bladder cancer, the relative risk associated with chewing was 1.7 and statistically significantly higher than 1.0. The authors estimated that tobacco chewing might account for 9 percent of the bladder cancer diagnoses in the area.

Although two studies did report elevated relative risks associated with smokeless tobacco use, on balance these studies provide little evidence to suggest that smokeless tobacco alters the risk of bladder cancer. It is possible that a small increase in risk has not been detected by the studies not reporting increases due to lack of statistical power.

Other Cancers

All other organs of the body are likely exposed to even lower concentrations of products of smokeless tobacco via the blood.

In a large prospective study in Norway, 16,713 individuals were interviewed to obtain information on the use of tobacco and alcohol and were followed up for development of pancreatic cancer *(40)*. Sixty-three persons in the cohort developed this neoplasm during a 10-year followup.

After controlling for cigarette smoking and alcohol consumption, a relative risk of 2.9 was observed in regular users of chewing tobacco or snuff (compared to nonusers). The 95-percent confidence limits of this value include 1.0. Risk was greater in regular users than former or occasional current users, and a trend of increasing risk with amount used was of borderline statistical significance ($P=.06$). The case-control analysis of the interview data from the TNCS *(24)* with respect to pancreas cancer is based on only 91 male cases (3 exposed to smokeless tobacco) and 85 female cases (none exposed); and although no increase in relative risk of pancreatic cancer in relation to smokeless tobacco was observed, the power of this study to detect such an increase is low.

Other cancer sites were found to be related to the use of smokeless tobacco in the case-control analysis of the interview data from the TNCS *(24)*. Relative risks for colon cancer at low and high levels of exposure were found to be 0.9 and 1.5 for men and 0.4 and 2.0 for women, respectively. Relative risks of cervical cancer in users of these two levels of exposure were 3.1 and 2.3. No studies have been conducted to confirm or refute these findings. In view of the large numbers of possible associations investigated, these results should be considered of value only in generating hypotheses for further investigation.

Summary

The epidemiologic studies showing an association between the use of snuff and oral cancers indicate that topical exposure of tissues to smokeless tobacco can cause cancers at the site of the exposure. Case reports of neoplasms developing in the ear and nose of individuals who used snuff at these sites raise the possibility that direct exposure may increase the risk in locations besides the oral cavity. Other tissues that come in contact with constituents of smokeless tobacco in more dilute concentrations include the linings of the esophagus, larynx (supraglotic portion), and stomach. Results of studies of cancers of these three sites in relation to smokeless tobacco are inconclusive; many are of limited power to detect small increases in risk and did not control for relevant, potentially confounding variables. However, some studies of these three cancers do show an increase in risk in relation to the use of smokeless tobacco. Constituents of smokeless tobacco can enter the bloodstream and some are excreted in the urine. The kidney and bladder are thus potentially exposed to these products and their metabolites but presumably in lower concentrations than are tissues of the upper aerodigestive tract. Evidence suggests that the risk of bladder cancer is not altered to any large extent in users of smokeless tobacco products, but results from studies of kidney cancer are inconsistent. Information regarding the risks of other cancers in relation to smokeless tobacco use is sparse.

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CHEMICAL CONSTITUENTS, INCLUDING CARCINOGENS, OF SMOKELESS TOBACCO

Chemical Composition of Smokeless Tobacco

To date, at least 2,500 known compounds have been identified in processed tobacco *(1)*. Besides polysaccharides and protein, tobacco contains *Nicotiana* alkaloids (0.5-5.0 percent), alkanes (0.1-0.4 percent), terpenes (0.1-3.0 percent), polyphenols (0.5-4.5 percent), phytosterols (0.1-2.5 percent), carboxylic acids (0.1-0.7 percent), aromatic hydrocarbons, aldehydes, ketones, amines, amides, nitriles, N- and O-heterocyclic compounds, chlorinated organic compounds, alkali nitrates (0.2-5.0 percent), and at least 30 metal compounds *(2,3)*.

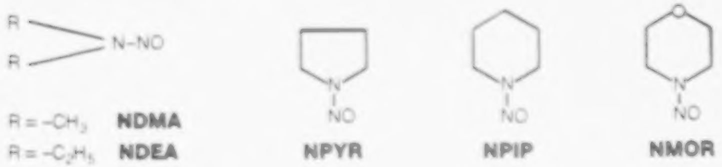
The most important habituating agent in tobacco is nicotine, the major representative of the alkaloids that constitute 0.5-5 percent of the leaf depending on the strain, variety, and agricultural practices that are employed during the tobacco cultivation. In total, the alkaloids are composed of 85 to 95 percent nicotine *(4)* and of other major alkaloids such as the secondary amines nornicotine, anatabine, and anabasine with lesser amounts of cotinine, myosmine, nicotyrine, 2,3′-dipyridyl, and N′-oxynicotine *(5)*.

Carcinogens in Smokeless Tobacco

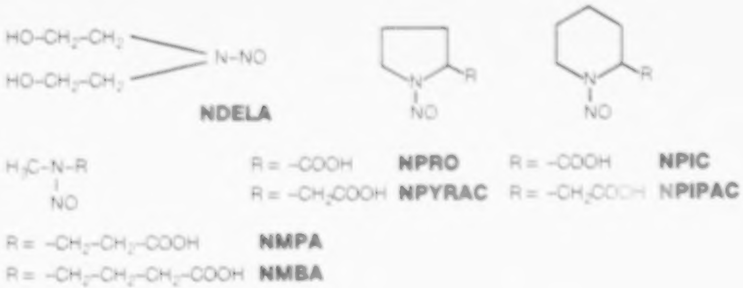
At present, three classes of carcinogens are known to occur in smokeless tobacco products: N-nitrosamines, polynuclear aromatic hydrocarbons (PAH), and polonium-210 (^{210}Po). Although chemical-analytical

FIGURE 1.—N-Nitrosamines in Smokeless Tobacco

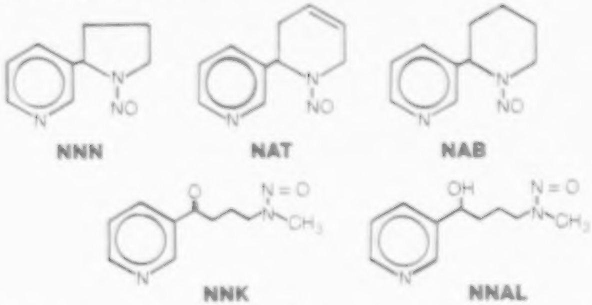
1. Volatile Nitrosamines


R
R
N–NO
R = –CH3 NDMA
R = –C2H5 NDEA
N
NO
NPYR
N
NO
NPIP
O
N
NO
NMOR

2. Nonvolatile Nitrosamines


HO–CH2–CH2
HO–CH2–CH2
N–NO
NDELA
N
NO
R
N
NO
R
H3C–N–R
NO
R = –COOH NPRO
R = –CH2COOH NPYRAC
R = –COOH NPIC
R = –CH2COOH NPIPAC
R = –CH2–CH2–COOH NMPA
R = –CH2–CH2–CH2–COOH NMBA

3. Tobacco-Specific Nitrosamines


N
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NAB
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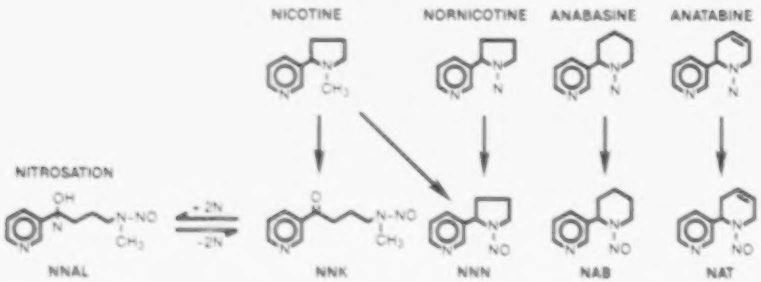
N=O
N
CH3
N
NNK
OH
N=O
N
CH3
N
NNAL

data are lacking, some smokeless tobacco mixtures contain or are suspected to contain traces of cadmium and nickel compounds *(6)*, formaldehyde, and coumarin, all of which are known animal carcinogens *(7,8)*.

N-Nitrosamines

Tobacco leaves contain an abundance of amines in the form of proteins and alkaloids. Tobacco also contains up to 5 percent nitrates and traces of nitrite. Thus there is the potential for the formation of N-nitrosamines from the nitrate, nitrite, and amines during the processing of smokeless tobacco products. In tobacco, we distinguish between volatile nitrosamines, nonvolatile nitrosamines, and tobacco-specific nitrosamines (figure 1). With the exception of some N-nitrosamino

FIGURE 2.—Formation of Tobacco-Specific Nitrosamines



acids, the nitrosamines in tobacco are animal carcinogens that are formed after harvesting of the tobacco during curing, fermentation, and/or aging. The N-nitrosamino acid, N-nitrosoproline, occurs in processed food and can also be formed in humans by endogenous nitrosation of proline. This nitrosamino acid is not carcinogenic on the basis of presently available data *(9-12)*. Table 1 summarizes the available data for the volatile nitrosamines in smokeless tobacco. Only one of the volatile nitrosamines, NDMA, has been found in U.S. looseleaf tobacco, but four nitrosamines have been found in American snuff. N-Nitrosomorpholine is formed during tobacco processing or aging from morpholine, a cyclic amine that is not known to occur in uncontaminated tobacco *(13,14)* but originates from packing materials and/or flavor additives. Table 2 lists the presently known nonvolatile nitrosamines in smokeless tobacco. N-Nitrosodiethanolamine (NDELA) in U.S. tobacco originates primarily from residues on tobacco leaves of the sucker-growth inhibitor maleic hydrazidediethanolamine (MH-30). Use of this formulation of the agricultural spray was banned in the United States in 1981, and the concentration of NDELA in smokeless tobaccos has markedly decreased since then *(14,15)*.

Figure 2 presents the formation of the tobacco-specific N-nitrosamines (TSNA) from the alkaloids. There is progressive nitrosation of the alkaloids during curing and processing and even during the shelf life of the commercial products *(16)*. Table 3 summarizes the presently available quantitative data for four out of five TSNA's that are present in smokeless tobacco. The nitrosamines are detectable in snuff and tobacco products from various parts of the world. Analyses of Swedish snuff brands manufactured between 1980 and 1985 have revealed a significant decrease of the levels of TSNA; such a trend has not been observed for U.S. snuff brands *(14,16,17)*. It has been suggested that the lowering of TSNA levels in Swedish snuff brands is due to better control of the bacterial content of the tobacco products. Reduced bacterial activity will probably reduce nitrite levels and, consequently, inhibit nitrosamine

TABLE 1.—Volatile Nitrosamines in Smokeless Tobacco (ppb)*

Product	NDMA	NPYR	NPIP	NMOR	Reference
U.S.					
Looseleaf†	ND - 380 (4)	ND - 1.2 (4)	ND (4)	ND - 2.5 (4)	*13,14,17,34*
Snuff	ND - 215 (26)	ND - 291 (16)	ND - 107 (16)	ND - 690 (26)	*13,14,17,20, 29,34-37*
Sweden					
Chewing Tobacco	ND - 0.6 (4)	0.9 - 3.7 (4)	ND (2)	ND - 0.8 (2)	*17,36*
Snuff	ND - 60 (53)	ND - 210 (27)	ND - 0.5 (37)	ND - 1.2 (53)	*14,17,36*
Canada					
Snuff	23 - 72.8 (2)	321 - 337 (2)			*14*
Denmark					
Chewing Tobacco	ND - 8.6 (6)	7.0 - 25.5 (6)	ND (4)	ND - 32.8 (6)	*17,36*
Norway					
Chewing Tobacco	37 - 220 (2)	84.0 - 280 (2)	2.8 - 15 (2)	28 - 37 (2)	*17*
India					
Chewing Tobacco	ND - 0.56 (4)	1.55 - 4.48 (4)		ND (4)	*14*
U.S.S.R.					
Nass‡	ND (4)	1.74 - 8.82 (4)		ND (4)	*14*

* Number in parentheses, number of samples analyzed.

† One sample also contained 8.6 ppb NDEA.

‡ Also contained ND - 69.6 NDEA *(14)*.

TABLE 2.—Nonvolatile Nitrosamines in Smokeless Tobacco (ppb)*

Tobacco Product	NDELA	NMPA	NMBA	NPRO	NPYRAC	NPIC	NPIPAC	Reference
U.S.								
Looseleaf	224 - 680 (3)			450 - 463 (2)				*13,14,34*
Snuff	160 - 6,800 (13)	1,250 - 7,420 (5)	120 - 2,240 (5)	500 - 50,900 (13)	ND - 2,000 (5)	ND - 6,100 (5)	ND - 1,500	*13-15, 34, 38,39*
Sweden								
Snuff	230 - 390 (8)	510 - 4,400 (12)	ND - 260 (12)	890 - 29,500 (12)	100 - 300 (5)	ND - 5,560 (12)	100 - 200 (5)	*14,15,38,40*
Canada								
Plug Tobacco	110 (1)			100 (1)				*14*
Snuff	1,180 - 2,720 (3)			8,800 - 16,600 (2)				*14*
Germany								
Plug Tobacco	50 (2)			500 - 700 (2)				*14*
Belgium								
Chewing Tobacco		1,600 (1)	100 (1)	3,300 (1)	200 (1)	100 (1)	200 (1)	*40*
U.S.S.R.								
Nass	40 (4)			ND - 180 (4)				*14*
India								
Chewing Tobacco	30 - 110 (4)			190 - 410 (4)				*14*

* Number in parentheses, number of samples analyzed.

TABLE 3.—Tobacco-Specific N-Nitrosamines in Smokeless Tobacco (ppb)*

Product	NNN	NNK	NAT	NAB	Reference
U.S.					
Looseleaf	620-8,200 (9)	ND-380 (4)	130-2,300 (5)	ND-140 (5)	*14,17,41,42*
Plug Tobacco	3,400-4,300 (3)				*43*
Snuff	1,600-135,000 (21)	100-13,600 (21)	1,560-338,000 (21)	10-6,700 (12)	*6,14,16,17,38,42,43*
Sweden					
Snuff	3,050-154,000 (34)	510-2,950 (34)	1,600-21,400 (34)	110-150 (19)	*14,16,17,38*
Plug Tobacco	350-2,090 (3)	ND-240 (3)	690-1,580 (3)	ND-100 (3)	*14,17*
Canada					
Snuff	50,420-79,100 (2)	3,200-5,800 (2)	152,000-170,000 (2)	4,000-4,800 (2)	*14*
Norway					
Snuff	13,000-29,000 (2)	2,700-3,900 (2)	9,100-16,000 (2)	1,000-2,400 (2)	*17*
Denmark					
Snuff	4,460-8,000 (3)	1,350-7,030 (3)	2,680-6,170 (3)		*16*
Chewing Tobacco	210-1,400 (4)	ND-210 (4)	300-2,800 (4)	ND-60 (4)	*17*
Germany					
Plug Tobacco	1,420-2,130 (2)	30-40 (2)	330-500 (2)	30-50 (2)	*14*
Snuff	6,080-6,700 (2)	1,500-1,540 (2)	3,920-4,370 (2)		*16*
U.S.S.R.					
Nass	120-520 (4)	20-130 (4)	32-300 (4)	8-30 (4)	*14*
India					
Chewing Tobacco	470-2,400 (5)	130-230 (4)	300-450 (4)	30-70 (4)	*14,41*
Belgium					
Chewing Tobacco	7,380 (1)	970 (1)	130 (1)		*38*

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* Number in parentheses: number of samples analyzed.

TABLE 4.—Estimated Exposure of U.S. Residents to Nitrosamines*

Source of Exposure	Nitrosamines	Primary Exposure Route	Daily Intake µg/Person	
Beer	NDMA	Ingestion	0.34	
Cosmetics	NDELA	Dermal Absorption	0.41	
Cured Meat:				
Cooked Bacon	NPYR	Ingestion	0.17	
Scotch Whiskey	NDMA	Ingestion	0.03	
Cigarette Smoking	VNA†	Inhalation	0.3	
	NDELA	Inhalation	0.5	
	NNN	Inhalation	6.1	16.2
	NNK	Inhalation	2.9	
	NAT+NAB	Inhalation	7.2	
Snuff Dipping‡	VNA	Ingestion	3.1	
	NDELA	Ingestion	6.6	
	NNN	Ingestion	75.0	164.5
	NNK	Ingestion	16.1	
	NAT+NAB	Ingestion	73.4	

* From the National Research Council *(18)*, amended by data for snuff dipping *(13)*. In addition, it has been established that upon inhalation of the air in cars with new leather upholstery daily exposure amounts to 0.50 µg of NDMA and 0.20 µg of NDEA *(18)*.

† VNA, NDMA + NEMA + NDEA + NPYR *(37)*.

‡ Brunnemann et al. *(13)*; average values from the leading five U.S. fine-cut tobaccos used for snuff dipping in 1981; assumed daily consumption 10 g/day of snuff. VNA = NDMA + NPYR + NMOR.

formation *(17)*. NNK and NNN are powerful carcinogens in mice, rats, and hamsters, NAB is moderately carcinogenic, and NAT is inactive in rats in doses up to 9 mmol/kg (table 3, page 82) *(3)*.

The daily exposure of an "average" snuff dipper to carcinogenic N-nitrosamines exceeds by at least two orders of magnitude the estimated exposure of U.S. residents to nitrosamines in products other than tobacco products (table 4) *(18,19)*. Furthermore, the concentrations of carcinogenic nitrosamines in snuff exceed very significantly the permissible limits for individual nitrosamines in consumer products (table 5).

During snuff dipping or chewing of tobacco, the TSNA's are extracted by the saliva. Consequently, the saliva of snuff dippers is reported to contain 5.0-420 ppb of NNN, up to 96 ppb of NNK, and 6.6-555 ppb of NAT *(16)*. The saliva analyses of Indian tobacco chewers showed the presence of 1.2-220 ppb of NNN, 3.2-51.7 ppb of NAT, and up to 2.3 ppb of NNK *(20,21)*. Recently, three additional TSNA's have been isolated from U.S. commercial snuff: 4-(methylnitrosamino)-1-(3-pyridyl)butanol-1 (NNAL), 4-(methylnitrosamino)-1-(3-pyridyl) butene-1 (NNO), and 4-(methylnitrosamino)-4-(3-pyridyl)butanol-1 (Red NNA) (figure 3) *(22)*. Additional amounts of TSNA's are most likely also formed by nitrosation processes that occur in the oral cavity during chewing *(19-21,23)*.

TABLE 5.—Permissible Limits for Individual N-Nitrosamines in Consumer Products

Product	Permissible Limit ppb (µg/kg)	Agency
Bacon (Meat)	5	USDA*
Beer	5	FDA†
Rubber Nipples of Baby Bottles	10	FDA‡

	Range of Individual Nitrosamines Present in Snuff Tobaccos ppb (µg/kg)	
NNN	5,800 - 64,000	Range in the leading 5 U.S. brands (1984-85)
NNK	100 - 3,100	
NAT	3,300 - 215,000	
NAB	200 - 6,700	
NDELA	160 - 6,800	Range in 13 U.S. brands (1980-1985)

* No "confirmable levels of nitrosamines" *(44)*

† Regulation set for N-nitrosodimethylamine *(45)*

‡ Regulation set for any individual volatile N-nitrosamine *(46)*

Polynuclear Aromatic Hydrocarbons

A number of naphthalenes have been identified in processed tobacco and especially in Latakia, which is flavor enriched by treatment with wood smoke *(24,25)*. While smoking tobaccos were found to contain 300-5,000 ppb of phenanthrene, 110-4,200 ppb of anthracene, 76-1,800 ppb of pyrene, 15-14,000 ppb of fluoranthene, and 8.5 ppb of benzo(a)pyrene (BaP) *(26,27)*, analyses of British snuff in 1957 showed levels of 260 ppb of pyrene, 335 ppb of fluoranthene, and 72 ppb of BaP *(28)*. In the five most popular snuff brands in the United States that were analyzed in 1985, BaP ranged from < 0.1 to 63 ppb *(29)*.

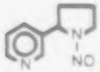
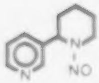
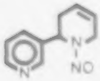
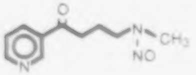
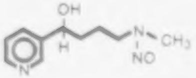
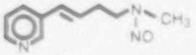
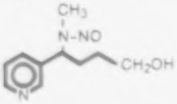
Polonium-210

This alpha-emitting element has long been incriminated as a human carcinogen *(30)*. The levels of ^{210}Po in dozens of U.S. and foreign cigarette tobaccos were between 0.1 and 1.0 pCi/g *(31)*. In recent samples of the five leading U.S. snuff brands, ^{210}Po ranged from 0.16 to 1.22 pCi/g *(29)*. It appears that ^{210}Po in tobacco leaves stems partially from certain types of fertilizers and airborne particles that are taken up by the trichomes (glandular hair) of the tobacco leaf *(31-33)*.

Summary

In processed tobacco, more than 2,550 chemical compounds have been identified. Among these are traces of known carcinogens such as

FIGURE 3.—Tobacco Specific N-Nitrosamines in Snuff
U.S. Brands, 1985

Nitrosamines		Relative Carcinogenicity in Rats*	Concentration in Snuff (ug/g) (Dry Weight)	
			A	B
NNN		+ + +	3.3	64
NAB		+	1.1	6.7
NAT		±	44	215
NNK		+ + +	1.8	3.1
NNAL†		+	0.3	0.14
NNO†		?	trace‡	trace‡
Red NNA		?	1.3	1.8

* + + + Tumors with 1 mmol/kg; + tumors with 9 mmol/kg; (for type of tumors induced see table 4, page 38); ± insignificant number of tumors with 9 mmol/kg; ? not tested.

† Isolated amounts only.

‡ < 0.01 ug/g.

PAH, ^{210}Po, and N-nitrosamines. The most prevalent organic carcinogens are the tobacco-specific N-nitrosamines that are formed from the *Nicotiana* alkaloids during the processing of tobacco leaves. Their concentrations in snuff exceed the levels of nitrosamines in other consumer products by over one hundredfold. During snuff dipping or chewing of tobacco, the nitrosation process continues within the mouth stimulated by oral bacteria.

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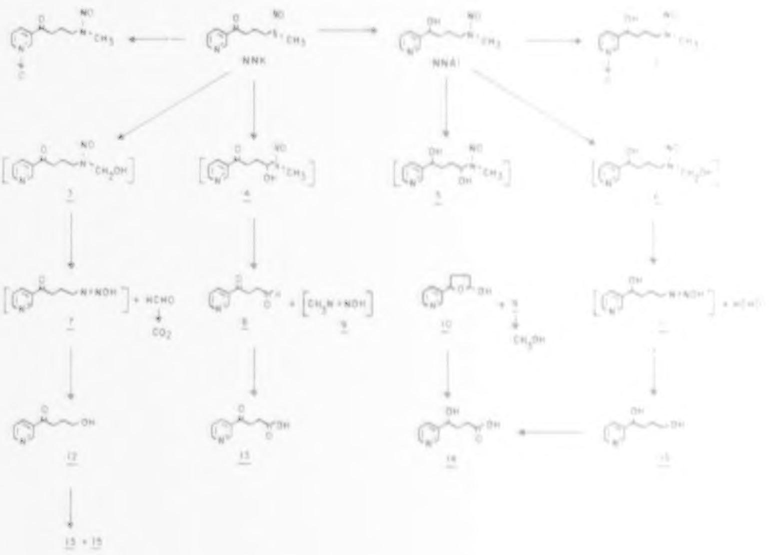
Abbreviations

BaP	Benzo(a)pyrene
NAB	N'-Nitrosoanabasine
NAT	N'-Nitrosoanatabine
ND	Not detected
NDEA	Nitrosodiethylamine
NDELA	Nitrosodiethanolamine
NDMA	Nitrosodimethylamine
NMBA	Nitrosomethylbutyric acid
NMOR	Nitrosomorpholine
NMPA	Nitrosomethylpropionic acid
NNAL	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N'-Nitrosonornicotine
NNO	4-(Methylnitrosamino)-1-(3-pyridyl)butene-1
NPIC	Nitrosopipecolic acid
NPIP	Nitrosopiperidine
NPIPAC	Nitrosopiperidine-acetic acid
NPRO	Nitrosoproline
NPYR	Nitrosopyrrolidine
NPYRAC	Nitrosopyrrolidine-acetic acid
PAH	Polynuclear aromatic hydrocarbons
$^{210}\mathrm{Po}$	Polonium-210
Red NNA	4-(Methylnitrosamino)-4-(3-pyridyl)-1-butanol
TSNA	Tobacco-specific nitrosamines

METABOLISM OF CONSTITUENTS OF SMOKELESS TOBACCO

The tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are quantitatively the major known carcinogens that are present in snuff and other types of smokeless tobacco. Molecular changes that are induced in the genetic material of tobacco chewers are most likely to arise from the metabolism of these two nitrosamines. Although present in similar quantities, N'-nitrosoanabasine (NAB) and N'-nitrosoanatabine (NAT) are less carcinogenic than NNK and NNN and are less likely to play an important role in the induction of oral cancer in man. Some snuff products contain considerable amounts of N-nitrosomorpholine (NMOR) and N-nitro-

FIGURE 1.—Metabolic Pathways of NNK

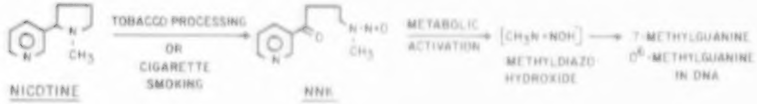


sodiethanolamine (NDELA); the former is a potent carcinogen. The levels of benzo[a]pyrene (BaP) and ^{210}Po in snuff tobacco are low compared to those of the nitrosamines (see previous section). This section will focus on the routes of metabolic activation of the compounds that are most likely to be involved in the induction of tumors that are related to snuff use—NNK, NNN, and NMOR.

Metabolism of NNK

The overall metabolic scheme for NNK, as determined by *in vivo* and *in vitro* studies in F-344 rats, Syrian golden hamsters, and A/J mice, is illustrated in figure 1 *(1-4)*. A key feature of this metabolic scheme is the conversion of NNK to the alpha-hydroxy intermediate *4*, which is unstable and undergoes spontaneous conversion to the keto aldehyde *8* and, most likely, methyl diazohydroxide *9*. The latter is a methylating agent that is well known for its ability to methylate DNA forming 7-methylguanine, 06-methylguanine, 4-methylthymidine, and a spectrum of other products *(5)*. Among these, 06-methylguanine, which is generated from precursors such as N-methylnitrosourea (NMU) or N-nitrosodimethylamine, has been unequivocally shown to be able to induce miscoding during DNA replication, and the resulting point mutation is sufficient to activate proto-oncogenes *(6,7)*. Many studies have demonstrated a correlation between 06-methylguanine persistence in replicating tissues and the initiation of the carcinogenic process, although it is clear in other cases that additional factors are also involved *(8,9)*.

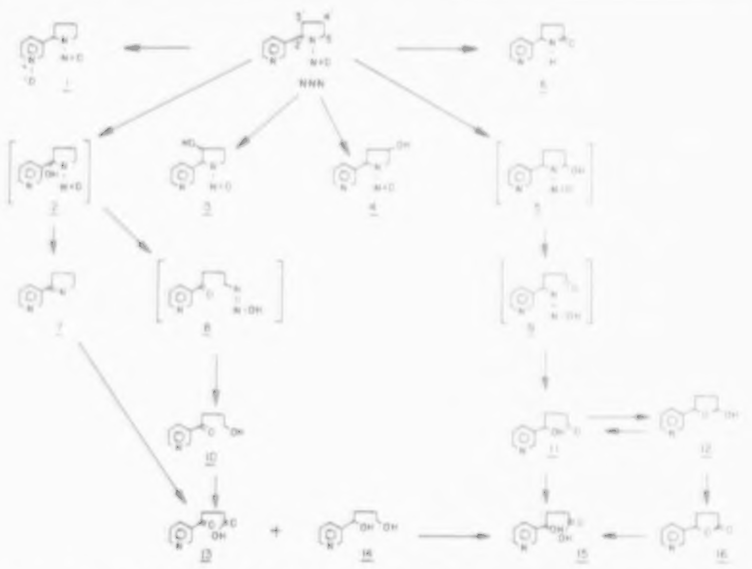
FIGURE 2.—Scheme Linking Nicotine to Formation of the Promutagenic DNA Adduct, O6-Methylguanine



Recent studies have demonstrated that NNK can methylate target tissue DNA of rats; 7-methylguanine and 05-methylguanine have been detected in the DNA of rat lung, nasal mucosa, and liver but not in the nontarget tissues, kidney, and esophagus *(10-14)*. These studies have also shown that, in the case of NNK, 06-methylguanine formation alone is not sufficient for tumor induction since persistent levels of 06-methylguanine in the lung were less than those observed upon treatment with equivalent quantities of N-nitrosodimethylamine, but the latter did not induce lung tumors *(13)*. It is clear from these, and related studies with NNN, that DNA adducts are also formed via pyridyloxobutylation or related processes. Regardless of the mechanism, it is significant that NNK causes DNA methylation; this creates a mechanistic link between nicotine, the habituating factor in tobacco, and 06-methylguanine formation in DNA, as illustrated in figure 2. Immunoassay methods are currently being developed to detect 06-methylguanine in the exfoliated oral cells of snuff dippers. Its presence can be inferred from the animal studies that are discussed above and by the demonstration that human tissues, including buccal mucosa, can metabolize NNK by alpha-hydroxylation *(15)*. In this respect, it is significant that injection of Syrian golden hamsters with the methylating agent MNU, combined with irritation of the buccal mucosa, resulted in the induction of oral cavity tumors *(16)*.

The pathway of NNK metabolism leading to the alpha-hydroxy intermediate 3 is also considered to be important in NNK carcinogenesis. This pathway gives rise to the electrophilic diazohydroxide 7. The properties of this intermediate have been investigated by using a model compound, 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (CNPB). Generation of 7 from CNPB is strictly analogous to the well-known ability of NMU to generate methyl diazohydroxide. Mutagenicity assays in *S. typhimurium* of CNPB have shown that it is more mutagenic than NMU *(17)*. Chemical model studies have demonstrated that it modifies the N2-position of deoxyguanosine *(18)*. This adduct and other adducts that may be formed from the diazohydroxide 7 and related intermediates are likely to play an important role in tumor induction by NNK. Autoradiographic studies have demonstrated that radioactivity from [carbonyl-14C]NNK is firmly bound to target tissues of rats and hamsters *(4,19)* and to tissues of the marmoset monkey *(20)*.

FIGURE 3.—Metabolic Pathways of NNN

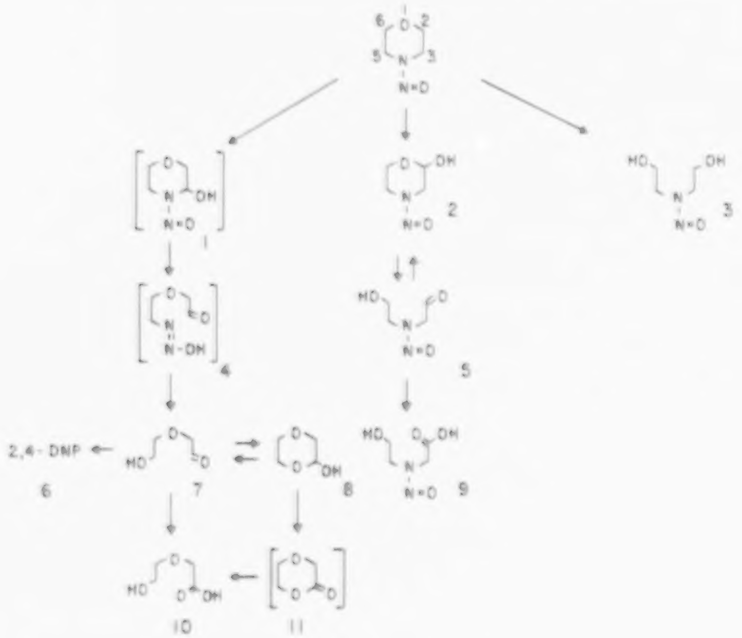


A third key feature of NNK metabolism is its rapid conversion in vivo and in cultured tissues from experimental animals and humans to its reduced form, NNA1, which has similar tumorigenic activity to that of NNK *(1,3,4,15,21)*. NNA1 is slowly metabolized as indicated in figure 1 and also by reconversion to NNK. Like NNK, it methylates DNA *in vitro* and *in vivo*. While the full details of the NNK-NNA1 equilibrium have not yet been elucidated, it is clear that NNA1 can act as a circulating source of NNK metabolites. It may play an important role in tissue-specific carcinogenesis by NNK.

Metabolism of NNN

Metabolic pathways of NNN are illustrated in figure 3. These pathways have been elucidated by *in vivo* and *in vitro* studies in rats, hamsters, and mice *(2,3,22-29)*. The stable metabolite NNN-1-N-oxide *(1)* has tumorigenic activity somewhat less than that of NNN but is still an effective carcinogen in F-344 rats *(30)*. Metabolism of NNN to the 2'- and 5'-hydroxy intermediates *2* and *5* constitutes a major pathway *in vivo* and *in vitro* in experimental animals, human liver microsomes *(31)*, and cultured human tissues, including buccal mucosa *(15)*. Of particular interest is the ability of two NNN target tissues, lingual mucosa and esophageal mucosa, to carry out preferential 2'-hydroxylation of NNN *(27,32)*. The intermediate that is formed by 2'-hydroxylation of NNN is diazohydroxide *8*, which is identical to that formed by methyl hydroxylation of NNN (7, figure 1). As described above, this intermediate is

FIGURE 4.—Metabolic Pathways of NMOR



highly mutagenic, and this or related intermediates appear to play an important role in carcinogenesis by both NNN and NNK. The intermediate *9* is significantly less mutagenic than *8* in *S. typhimurium (33)*, and various lines of evidence indicate that it is less important in NNN tumorigenesis than is *8 (33,34)*. Autoradiographic studies have demonstrated that radioactivity from [2'-^{14}C]NNN is bound to tissues of mice, rats, and marmoset monkeys *(20,35-37)*. Immunoassays are currently being developed for the putative DNA adducts that are produced by 2'-hydroxylation of NNN and methyl hydroxylation of NNK; it will be important to assess the levels of these adducts in the exfoliated oral cells of snuff dippers. Their levels may relate to the susceptibility of individuals to the effects of smokeless tobacco. The metabolic pathways that lead to these intermediates can be affected by alcohol consumption and dietary components *(32,38-43)*.

Metabolism of NMOR

The metabolic pathways of NMOR are illustrated in figure 4. These have been elucidated by *in vitro* and *in vivo* studies in rats *(44-47)*. Structure activity studies had shown that 3-hydroxylation of NMOR, leading to intermediate *4*, was likely to be important in NMOR carcinogenesis *(48)*. This pathway could result in the formation of glyoxal-deoxyguanosine adducts *(49)*; 2-hydroxylation of NMOR also occurs, giving the mutagenic product *2*. The latter also forms glyoxal-deoxyguanosine adducts *(50)*. These adducts, which are likely to have miscoding properties, also should be present in the DNA of snuff dippers since human tissues are capable of metabolizing NMOR *(51)*.

Summary

Persuasive evidence exists that the carcinogenic nitrosamines that are present in high quantities in snuff and other forms of smokeless tobacco are metabolized by target tissues of experimental animals and by human tissues to intermediates that can modify the genetic material of the cell.

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EXPERIMENTAL STUDIES INVOLVING EXPOSING LABORATORY ANIMALS TO SMOKELESS TOBACCO OR ITS CONSTITUENTS

This section reviews bioassays evaluating the carcinogenicity in animals of smokeless tobacco and its constituents, particularly the tobacco-specific nitrosamines (TSNA) described in the section on the chemical constituents of smokeless tobacco. The bioassays involved multiple routes of administration of chewing tobacco, snuff, or extracts of these products and of several TSNA.

Studies of chewing tobacco, snuff, and TSNA are summarized in tables 1 to 3 respectively, with comments on the individual investigations provided below.

Bioassays With Chewing Tobacco

Oral Administration

An alcohol extract of Indian chewing tobacco diluted 1:50 (group 1) or 1:25 (group 2) was gavage-fed to male Swiss mice over 15 to 20 months. In another group of mice, a mixture of the tobacco extract with standard laboratory diet was administered over 21 to 25 months (group 3). This treatment produced tumors in 8 of 15 mice at risk in group 1, including 5 mice with lung tumors and 2 with liver tumors; 4 of 10 mice at risk in group 2 developed lung and liver tumors. The feeding experiment (group 3) resulted in 8 of 10 mice with tumors, specifically 4 with tumors of the lung and 4 with liver tumors. Despite the high toxicity of the tobacco extracts and certain short-comings of the methodology, these assays indicate that the extract of chewing tobacco is carcinogenic in mice *(1)*.

Application to the Oral Mucosa and Cheek Pouch

Three different extracts of an Indian chewing tobacco were applied daily for up to 18 months to the buccal mucosa of strain A and Swiss mice. No excess of tumors was observed *(2)*. The oral mucosa of a group of weanling Wistar rats was painted twice weekly with a 2-percent alkaloid-free extract of an Indian chewing tobacco. No tumors were observed at the application site even though applications were continued throughout the lifespan of the rats *(3)*.

TABLE 1.—Bioassays for Carcinogenic Activity of Chewing Tobacco or Chewing Tobacco Extracts*

Route of Application	Species, Sex	Test Material and Dose	Duration of Exposure (Months)	Fraction of Animals With Tumors: Exposed	Fraction of Animals With Tumors: Controls	Reference
Oral-intubation	mice, M	extract diluted 1:25 diluted 1:50	4/1/2 15-20	4/10† lung adenocarcinoma 8/15† and liver carcinoma	0/20	*1*
Oral-feeding	mice, M	0.2% extract in diet	21-25	8/10† lung adenocarcinoma	1/20	*1*
Skin-topical	mice, M+F	DMSO extract (dose ?)	21-22	0/10 0/7		
Oral-swabbing	mice, M+F	extracts applied daily, dose not given	up to 18	no excess tumors compared to controls		*2*
Oral-swabbing	rats (NS)‡	2% alkaloid-free extract, dose not given- + lime	Lifespan	0/10 0/12	0/10 0/14	*3*
Oral-pouch implantation	hamsters (NS)	2-cm^3 plug	up to 30	0/50		*7*
Oral-pouch	hamsters (NS)	DMSO-extract three times weekly	18-24	0/12	0/7	*5*
Oral-pouch swabbing	hamsters, M	DMSO-extract three times weekly, dose not given	5	0/12	0/11	*4*
Oral-pouch swabbing	hamsters, F	2% tobacco extract in water, twice daily application	6	3/17	0/10	*6*
Subcutaneous injection	mice (NS)	2% tobacco extract partially or completely free of alkaloids, 25 solution once a month	10-23	1/17 squamous-cell carcinoma (site not specified)		*8*

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* Abbreviation: DMSO, dimethyl sulfoxide.

† Animals at risk.

‡ (NS) = not stated.

TABLE 2.—Bioassays for Carcinogenic Activity of Snuff or Snuff Extracts*

Route of Application	Species, Sex	Test Material and Dose	Applications	Duration of Exposure (Months)	Fraction of Animals With Tumors		Reference
					Exposed	Controls	
Oral-feeding	Hamsters, M	S, 20% of diet	Once daily	24	0/100†	0/100	*17*
Lips-painting	Mice, M	SE, dose not given	3 times daily	2	0/20	0/20	*18*
Oral-swabbing	Rats, M	SE+H	0.5 ml daily	2	0/20	0/20	*18*
		SE (approx. 30%)	0.5 ml daily	up to 30	0/30	1/21 (lung adenoma)	*20*
		SE (approx. 30%) + (NNN+NNK)	0.5 ml daily	up to 30	5/30 (3 papilloma in oral cavity, 2 lung adenoma)	1/21 (lung adenoma)	*20*
		NNN+NNK	0.5 ml daily	up to 30	13/30 (8 papilloma in oral cavity, 5 lung adenoma)	1/21 (lung adenoma)	*20*
Lip canal-instillation	Rats, F	S	200 mg twice daily	9-22	1/42† (oral carcinoma)	0/20	*21*
		S	200 mg twice daily	18	1/10 (oral carcinoma)	0/10	22
		H	200 mg	18	0/7	0/10	22
		S+H	twice daily	18	2/7 (2 oral carcinoma)	0/10	22
Lip canal-instillation	Rats, M	S	50 mg daily	up to 30	3/32 (papilloma and 1 carcinoma in test canal, 1 oral papilloma)	0/10	*20*
		S+Se	50 mg daily	up to 30	1/32 (oral papilloma)	0/10	*20*
		ES	50 mg daily	up to 30	2/21 (oral papilloma)	0/10	*20*
Cheek pouch-instillation	Hamsters (NS)‡	S	10 ml paste once	up to 30	0/50	0/50	*7*
		S	?	6	0/25		*27*
		H	?	6	0/25		*27*
		S+H	?	6	11/25 (papilloma and carcinoma of the oral cavity)		*27*
Subcutaneous injection	Rats, M+F	SE	50 mg, 84 weekly applications	26	0/82	0/82	*28*
	Rats (NS)	TE	45 mg, 70 weekly applications	21 ± 4	18/75	1/75	*29*

* Abbreviations: ES, extracted snuff; H, infected with herpes simplex virus; NNK, 4-(methylnitrosamino)-1-(-3-pyridyl)-1-butanone; NNN, N'-nitrosonornicotine; S, snuff; SE, snuff extract.

† No tumors of the oral cavity, esophagus, nasopharynx and larynx; all other tumors nearly identical to those in control animals.

‡ (NS) = not stated.

TABLE 3.—Carcinogenicity of Tobacco-Specific Nitrosamines*

Nitrosamine	Species and Strains	Route of Application	Principal Target Organs	Dose
NNN	A/J mouse	i.p.	lung	0.12 mmol/mouse
	F344 rat	s.c.	nasal cavity esophagus	0.2-3.4 mmol/rat
		p.o.	esophagus nasal cavity	1.0-3.6 mmol/rat
	Sprague-Dawley rat	p.o.	nasal cavity	8.8 mmol/rat
	Syrian golden hamster	s.c.	trachea nasal cavity	0.9-2.1 mmol/hamster
NNK	A/J mouse	i.p.	lung	0.12 mmol/mouse
	F344 rat	s.c.	nasal cavity lung, liver	0.1-2.8 mmol/rat
	Syrian golden hamster	s.c.	trachea, lung, nasal cavity	0.9 mmol/hamster 0.005 mmol/hamster
NAT	F344 rat	s.c.	none	0.2-2.8 mmol/rat
NAB	F344 rat	p.o.	esophagus	3-12 mmol/rat
	Syrian golden hamster	s.c.	none	2 mmol/hamster
NNA	A/J mouse	i.p.	none	0.12 mmol/mouse

* Hoffmann and Hecht *(11)*.

A group of 12 male Syrian golden hamsters received topical applications on the buccal mucosa of a dimethyl sulfoxide (DMSO) extract of an Indian chewing tobacco three times weekly for 21 weeks. None of the treated hamsters developed tumors in the oral mucosa; however, 8 of 12 treated animals had leukoplakia. These changes were not seen in the oral mucosa of the animals treated with DMSO alone *(4)*. In another bioassay, 12 male hamsters received applications to the cheek pouch of a DMSO extract of Indian chewing tobacco three times weekly over their entire lifespan. Tumors were not observed in the treated group or the control group *(5)*. When 1 mg of a paste made of a chewing tobacco extract was applied topically to the mucosa of the cheek pouches twice daily over a 6-month period, and animals were maintained without further treatment for another 6 months, the incidence of hyperplasia in the buccal pouches was 17.6 percent, that of dysplasia was 29.4 percent, and that of squamous cell papilloma or carcinoma was 17.6 percent in 17 hamsters. There were no tumors in the 20 control animals *(6)*.

Fifty hamsters received implantations of a 2 cm^3 plug of chewing tobacco in their cheek pouches. The opening of the cheek pouch was ligated and the animals were observed for 18 months. After 13 months, 21 of 50 animals had survived. No tumors were recorded upon termination of the assays *(7)*.

Although the studies cited above had some inherent weaknesses due to short application time or low dose, it appears, nevertheless, that both the oral mucosa of rats and the cheek pouches of Syrian golden hamsters are relatively resistant to the carcinogenic activity of the extracts of chewing tobacco.

Subcutaneous Application

Seventeen C57 black mice were subcutaneously injected with 1 ml of a 2-percent solution of either partly or completely alkaloid-free extracts of an Indian chewing tobacco once a month for 1 to 24 months. One squamous carcinoma at an unspecified site developed in one mouse receiving the partly alkaloid-free extract *(8)*.

Skin Application

A large number of studies have been published regarding the tumorigenicity on mouse skin of various extracts of chewing tobacco. Most of these bioassays failed to produce skin tumors. The negative results appear to be due primarily to the low dose applied or the short duration of the applications *(9,10)*. The negative results indicate also that the concentrations of TSNA and PAH in these extracts do not suffice to induce tumors upon topical application *(11)*. However, the application of methanol or DMSO extracts of cigarette tobacco induced a low but significant number of benign tumors in the skin of CAF1 and Swiss mice when these extracts were applied three times weekly for up to 24 months to the shaved backs of the mice *(12,13)*. A number of studies have reported tumor-promoting activity of the extracts of chewing tobacco when these were applied to mouse epidermis previously treated with a tumor initiator *(8,12,14-16)*. The bioassay data with chewing tobacco are summarized in table 1.

Bioassays With Snuff

Oral Administration

For 2 years, 50 male BIO 15.16 and 50 male BIO 87.20 hamsters were each maintained on a standard diet containing 20 percent moist, fresh snuff. Controls consisted of 50 male BIO 15.16 hamsters and 50 male BIO 87.20 hamsters on a diet containing 20 percent cellulose (of caloric value similar to the snuff-containing diet). The spectrum of tumors observed was nearly identical in both groups. Hamsters of both strains gavaged 60 times with 5 mg of the carcinogen 3-methylcholanthrene (MC) had a significantly increased incidence of both benign and malignant tumors of the forestomach and large intestine. Hamsters of the BIO 87.20 strain also had an increased incidence of stomach cancers while the BIO 15.16 strain developed tumors of the skin. To assay the cocarcinogenic activity of snuff, 50 hamsters of each strain received the diet containing 20 percent snuff plus 50 times 0.5 mg of MC. Compared to the control group (diet containing 20 percent cellulose), the tumor yield was not increased in the two experimental groups indicating a lack

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of carcinogenic activity as well as of cocarcinogenic activity of the snuff in this setting *(17)*.

Application to the Lip, Oral Mucosa, or Cheek Pouch

The upper lips of 20 male BALB mice were painted 3 times a day for 5 days weekly over a 2-month period with a concentrated water extract of snuff (group 1). In another group of 20 male mice, the upper lips were inoculated with herpes simplex virus type 1 (HSV-1) and were subsequently painted with a concentrated snuff extract for 2 months (group 2). A control group of 20 male mice received inoculation of the upper lips with HSV-1 and painting with water (group 3). Two months' exposure to snuff extract (group 1) or HSV-1 inoculation (group 3) alone did not induce dysplasia in the epithelium of the labial mucosa, while HSV-1 inoculation combined with painting of snuff extract produced epithelial dysplasia and other histomorphologic changes *(18)*.

In respect to this and other studies in which animals are infected with herpes virus in addition to treatment with snuff extracts, it should be noted that 20 to 40 percent of the U.S. population have periodic occurrences of labial herpes *(19)*.

Male F344 rats were treated for up to 30 months by swabbing the oral cavity with either a concentrated water extract of snuff (group 1; 13.2 μg NNN and 2.8 μg NNK per milliliter snuff extract solution), snuff extract enriched with the tobacco-specific nitrosamines NNN and NNK (group 2; 148 μg NNN and 30 μg NNK per milliliter snuff extract solution), NNN and NNK alone in concentrations corresponding to those applied in group 2 (group 3; 135 μg NNN and 27.6 μg NNK per milliliter test solution), or with water alone (group 4). Groups 1, 2, and 3 consisted of 30 male rats each and group 4 (control) of 21 rats. The incidence of tumors in groups 1 and 2 was not significantly increased over that in the control group. In the group of 30 rats treated with NNN and NNK alone, 8 animals had oral tumors (6 papillomas in the cheek, 4 papillomas in the hard palate, and 1 papilloma of the tongue), and 4 animals had lung carcinoma. This study indicates that snuff contains carcinogenic N-nitrosamines; however, when they are being tested in an admixture with other components in the water extract of snuff, their carcinogenic activity may be suppressed *(20)*.

A group of 21 male and 21 female Sprague-Dawley rats were treated with snuff placed in a surgically created canal in the lower lip. Approximately 0.2 g of a standard Swedish snuff (pH 8.3) was given twice daily 5 days per week for 9 to 22 months. The mean retention time of the snuff in the canal was 6 hours, and the estimated daily dose was 1 g of snuff/kg b.w. Using the same methodology, another group of 5 male and 5 female rats was treated with alkaline snuff in the surgically created canal (pH 9.3). One of the 42 rats treated with regular snuff developed a squamous carcinoma in the oral cavity after 8.5 months. The exposure to the regular snuff resulted in mild to moderate hyperplasia of the epithelium,

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hyperorthokeratosis, and acanthosis. Among rats exposed to snuff for 18 to 22 months, 16 of 42 showed vacuolated cells penetrating deeper into the epithelium with hyperplastic and atropic lesions. Rats exposed to alkaline snuff differed little from those in the group treated with regular snuff. Outside the area of treatment, squamous cell hyperplasia of the forestomach was found in rats exposed to snuff for 18 to 22 months *(21)*.

In another bioassay using the same methodology as described by Hirsch and Johansson *(21)*, the surgically created canal in the lower lip of F344 rats was filled five times each week over 28 months with either U.S. snuff (average 0.2 g per application; n=30), snuff enriched with its own water extract (n=30), or the extracted residue of snuff (n=21). Ten rats with the surgically created lip canal, and otherwise untreated, served as controls. The incidence of nonspontaneous tumors in each group was the following: rats treated with snuff had one squamous carcinoma of the oral cavity, one squamous cell papilloma of the hard palate, and one meningioma; treatment with enriched snuff led to one squamous cell papilloma of the floor of the mouth and one nasal olfactory tumor; treatment with extracted snuff induced one squamous cell papilloma of the hard palate. There were no tumors in the control group *(20)*.

Four groups of female Sprague-Dawley rats with surgically created canals in the lower lip, received the following treatments beginning at 3 months of age: group 1 was infected with herpes simplex virus type 1 (HSV-1) by scarification and topical application followed 10 days later by administration of snuff into the canal morning and night on 5 days per week; group 2 was infected with virus and received no other treatment; group 3 was sham-infected with sterile saline followed by snuff treatment; and group 4, not given virus or snuff, served as controls. The HSV-1 infection was repeated once after a 1-month interval, and snuff treatment was continued for 18 months after which time all animals were killed. Three animals in each of groups 1 and 2 died from encephalitis shortly after the second infection with HSV-1. Squamous-cell carcinomas of the oral cavity developed in two of seven rats, and a retroperitoneal sarcoma was seen in one of seven rats exposed to HSV-1 plus snuff. In the group exposed to snuff alone, 1 of 10 animals developed a squamous carcinoma of the anus and 1 of 10 a retroperitoneal sarcoma *(22)*.

In several studies, various forms of snuff were installed in the cheek pouches of Syrian golden hamsters for up to 20 months. The application of snuff did not lead to the induction of tumors in the cheek pouches nor at any other site of the oral cavity in any of these studies even though malignant tumors were induced in the oral cavity with high doses of 7, 12-dimethylbenz(a) anthracene and 3-methylcholanthrene *(7,23-26)*.

In an assay for the joint action of HSV and snuff, the buccal pouches of 125 Syrian hamsters were inoculated with HSV-1, HSV-2, or culture medium. The control and HSV inoculations were done once a month for 6 consecutive months. Then 25 hamsters with HSV-inoculated pouches received installations of commercial snuff twice daily into both the right

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and left pouches. One month after the last HSV inoculation and 6 months after continuous snuff application, the assay was terminated. The buccal pouches were removed for histopathologic examination. Neither the application of snuff to the cheek pouches nor HSV infection alone induced neoplastic changes in hamster buccal pouches. However, HSV infection in combination with snuff resulted in epithelial dysplasia and in squamous carcinoma of the buccal pouches in 11 out of 25 hamsters *(27)*. This investigation provides the strongest evidence to date that snuff may increase cancer risk in animals; however, full evaluation is precluded since the findings have been published only in abstract form.

Subcutaneous Administration

A Swedish snuff was extracted with 60-percent alcohol and resulted in 18-percent dry extract, which was injected subcutaneously into rats with 70-percent ethanol and tri-n-caprylin (1:1) as vehicle. The rats received a total dose of 4.2 g of extract with 84 weekly doses of 50 mg of extract. No tumors were observed at the area of injection *(28)*. This result is quite different from an earlier one by the same investigators in which an alcohol extract from cigarette tobacco (20-percent yield) was injected into 75 rats with 70-percent alcohol and glycerol as solvent (1:3). Per week, 45 mg extracts were injected until the total dose amounted to 3.2 g/rat. After 25 months, 18 of 75 rats had developed malignant tumors, primarily sarcomas at the injection site *(29)*. The bioassay data with snuff are summarized in table 2.

Bioassays With Constituents of Smokeless Tobacco

At least three types of carcinogens occur in smokeless tobacco: polynuclear aromatic hydrocarbons (PAH), polonium-210 (^{210}Po), and N-nitrosamines. One of the PAH identified in smokeless tobacco, benzo(a)pyrene (up to 72 ppb), has long been recognized as an animal carcinogen *(18,24,30)*. Levels of ^{210}Po in processed tobacco amount to 0.1-1.0 pCi per gram and to 0.18-1.22 pCi/g in commercial U.S. snuff products. Ionizing radiation can cause multiple types of cancer in animals and humans raising the possibility that the alpha-radiation of ^{210}Po may contribute to the carcinogenic potential of smokeless tobacco and especially snuff *(31,31)*.

Three groups of N-nitrosamines have been identified in smokeless tobacco. All of the 4 volatile nitrosamines thus far identified are carcinogenic in animals *(33)*. These are nitrosodimethylamine (0 to 215 ppb), nitrosopyrrolidine (0 to 291 ppb), nitrosopiperidine (0 to 107 ppb), and nitrosomorpholine (0 to 690 ppb). Seven nonvolatile nitrosamines have also been identified in smokeless tobacco. Of these, only nitrosodiethanolamine (30 to 6,800 ppb) is a known carcinogen in mice, rats, and hamsters *(33)*. Swabbing of the oral cavity of 20 male and 20 female hamsters with solutions of these agents three times weekly for 45 weeks

(20 mg per application) induced tumors of the nasal cavity in 17 animals, tumors of the trachea in 6, and a tumor of the larynx in 1 of the hamsters *(34)*.

The most abundant carcinogens in smokeless tobacco yet identified are the tobacco-specific nitrosamines (TSNA). These are formed during the processing of tobacco from its alkaloids. So far, seven TSNA have been identified in smokeless tobacco. Of these, N′-nitrosonornicotine (NNN; 470-135,000 ppb) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; 30-13,600 ppb) are powerful carcinogens in mice, rats, and hamsters (table 1; 1,9). Table 3 summarizes results from bioassays administering TSNA to test animals. A variety of tumors were produced, particularly in the esophagus, nasal cavity, and lung. In a recently completed investigation, daily swabbing for up to 30 months of the oral cavity of F344 rats with a saline solution containing 135 ppm NNN and 28 ppm NNK led to the development of benign oral tumors in 8 and lung carcinoma in 4 of 30 rats. Neither oral tumors nor tumors of the lung were observed in the negative control group *(20)*. This study suggests that NNN and NNK may be tumorigenic at the site of exposure as well as systemically. Full evaluations of these results are precluded, however, since the original manuscript is now under journal review and not published.

It is noteworthy that some of the bioassays indicated that relatively low doses of the TSNA could induce tumors. In hamsters, a total dose of only 0.2 mmol/kg of NNK induced a significant incidence of tumors *(35)*, whereas in F344 rats, 60 subcutaneous injections of a total dose of 20 mg (0.33 mmol/kg) of NNK induced tumors of the liver in 10, tumors of the lung in 13, and tumors of the nasal cavity in 6 of 30 rats. Subcutaneous applications to 27 rats of the same molar dose (0.33 mmol/kg) of nitrosodimethylamine resulted in 6 animals with tumors of the liver and 1 rat with a tumor of the nasal cavity *(36)*. For NNN, high tumor incidences were produced in F344 rats by a total dose of 1.0 mmol/kg *(37)*. Based on daily use for 30 years of 10 g of snuff containing 3.1 ppm of NNK, the estimated NNK exposure of a snuff dipper would be approximately 0.02 mmol/kg. Exposure to NNN from the same brand would be 0.4 mmol/kg (figure 3, chapter 2). Hence, the bioassays indicate that exposures in the dose range actually experienced by long-term snuff dippers induce tumors in animals. This is a distinctive and potentially important finding, since for most chemical carcinogens their carcinogenicity was detected following exposure at doses much higher than usually received by humans.

Of the other five TSNA, besides NNN and NNK, N′-nitrosoanabasine (NAB; 10-6,700 ppb) and 4(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; 140-300 ppb) were moderately active carcinogens, and N-nitrosoanatabine (NAT; 300-338,000 ppb) was inactive when tested at the low dose level of 9 mmol/kg *(9,38)*.

Recently, two additional TSNA have been identified in snuff: 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanone (1,300-1,800 ppb) and 4-(methylnitrosamino)-(3-pyridyl)butene-1 (10 ppb; 6). These two nitrosamines have not yet been tested for carcinogenicity.

Mutagenicity Assays and Other Short-Term Tests

Chewing Tobacco

Nicotiana rustica is a tobacco variety that is widely cultivated and used throughout India. Its ethanol extracts induced mutations in *Salmonella typhimurium* TA98 and in V79 cells of Chinese hamsters. The addition of S9 liver homogenate from Aroclor-pretreated rats enhanced the mutagenic effect. No mutations were induced in TA100, TA1535, or TA1538 in the presence of the S9 homogenate. This ethanol extract of tobacco also induced micronuclei in bone marrow cells of Swiss mice *(1,39,40)*.

An ethyl acetate extract of Indian chewing tobacco induced sister chromatid exchange (SCE) in human lymphocytes and in a human lymphoblastoid cell line. In the latter system, S9 rat liver homogenate enhanced the effect. When the tobacco extract was tested in the absence of the S9 homogenate it did not induce ouabain-resistance in Chinese hamster V79 cells. The same extract, another ethyl acetate extract, and an ethanol extract of tobacco induced cell transformation in Syrian hamster embryo cells *(41,42)*.

The incidence of micronucleated oral mucosa cells in 27 Indians using khani chewing tobacco was 2.2 percent (0.8-4.9 percent). The incidence of micronuclei in exfoliated cells of nonchewers of similar ethnic backgrounds and dietary habits was 0.47 percent (0.0-0.9 percent) *(43)*.

Snuff

The residue of organic solvent extracts from a U.S. commercial snuff was dissolved in DMSO and tested for the induction of SCE's in human peripheral lymphocytes. The organic snuff extract induced significant SCE's with a 0.05 percent concentration in lymphocytes of one of three donors, with a 0.15 percent concentration in lymphocytes in two of three donors, and with a 0.5 percent concentration in lymphocytes of all three donors *(44)*.

Tobacco-Specific N-Nitrosamines (TSNA)

Of the seven TSNA so far identified in smokeless tobacco, only NNN and NNK were also tested for genotoxicity in short-term tests. In the presence of a liver microsomal preparation from Aroclor-induced rats, NNN and NNK caused dose-dependent mutations in *Salmonella typhimurium* TA100 and TA1535. Increased mutation frequencies were observed in the case of NNN at 2.5 µmol and at 5.65 µmol/plate and in the case of NNK at 1.4 µmol/plate (45-47).

NNN and NNK at 10-3 and 10-2 molar concentration each induced unscheduled DNA synthesis in freshly isolated hepatocytes from adult rats *(48)*.

Summary

Chewing tobacco and extracts from various chewing tobaccos have been tested by oral administration in mice, topical application to the oral mucosa of mice, rats, and hamsters, and by subcutaneous administration and skin application to mice. The investigations failed to demonstrate significantly increased tumor production. Short application times and low-dose exposures, however, limit the evaluation of the carcinogenicity of chewing tobacco or its extracts. Bioassays of snuff have likewise generally shown no excess cancer, although some experiments suggest that it may cause oral tumors in rats and hamsters that are infected with herpes simplex virus. Among the chemical components of snuff, the tobacco-specific nitrosamines NNN and NNK are powerful carcinogens. The doses of NNN and NNK that produce tumors in experimental animals are close to the doses estimated from lifetime exposure among human snuff dippers.

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CONCLUSIONS

1. The scientific evidence is strong that the use of smokeless tobacco can cause cancer in humans. The association between smokeless tobacco use and cancer is strongest for cancers of the oral cavity.

2. Oral cancer has been shown to occur several times more frequently among snuff dippers than among nontobacco users, and the excess risk of cancers of the cheek and gum may reach nearly fiftyfold among long-term snuff users.

3. Some investigations suggest that the use of chewing tobacco also may increase the risk of oral cancer.

4. Evidence for an association between smokeless tobacco use and cancers outside of the oral cavity in humans is sparse. Some investigations suggest that smokeless tobacco users may face increased risks of tumors of the upper aerodigestive tract, but results are currently inconclusive.
5. Experimental investigations have revealed potent carcinogens in snuff and chewing tobacco. These include nitrosamines, polycyclic aromatic hydrocarbons, and radiation-emitting polonium. The tobacco-specific nitrosamines N-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone have been detected in smokeless tobacco at levels 100 times higher than the regulated levels of other nitrosamines found in bacon, beer, and other foods. Animals exposed to these tobacco-specific nitrosamines, at levels approximating those thought to be accumulated during a human lifetime by daily smokeless tobacco users, have developed an excess of a variety of tumors. The nitrosamines can be metabolized by target tissues to compounds that can modify cellular genetic material.
6. Bioassays exposing animals to smokeless tobacco, however, have generally shown little or no increased tumor production, although some bioassays suggest that snuff may cause oral tumors when tested in animals that are infected with herpes simplex virus.

RESEARCH NEEDS

It has been established beyond reasonable doubt that smokeless tobacco use can increase the risk of cancer. The experimental and epidemiologic evidence is strongest for the association between oral cancer and the chronic use of snuff. Additional studies are needed to determine whether the patterns of risk differ according to the form of smokeless tobacco, including research evaluating cancer risks that are associated with chewing tobacco and dry versus moist snuff, and to quantify further the levels of risk in relation to differing levels of smokeless tobacco exposure.

The influence of smoking, alcohol, and other factors (including viral exposures) on the smokeless tobacco-associated risk of oral cancer also should be explored further with an emphasis on detecting possible interactions between these factors and smokeless tobacco.

Inhaled snuff may increase the risk of nasal carcinoma. The feasibility of initiating studies in areas where snuff sniffing is common should be ascertained, and studies should be launched to confirm and quantitate this possible relationship.

There have been few studies of smokeless tobacco and esophageal, laryngeal, and gastric cancers. These investigations have provided equivocal results, but in the aggregate, their findings raise the possibility of some increase in risk among smokeless tobacco users. Additional case-control studies of these neoplasms should be encouraged. These studies should be large enough to assess the risks that are associated with smokeless tobacco use while controlling for the potential confounding effects of smoking, alcohol, and other risk factors.

Isolated reports have associated smokeless tobacco with cancers of the cervix, pancreas, and other anatomic sites. Investigators with existing data from case-control studies of these neoplasms should be encouraged to perform analyses to determine whether associations with smokeless tobacco exist. Similarly, existing data from cohort studies with information on smokeless tobacco use should be analyzed. Reports from two relatively large cohort studies have been published only as abstracts. These should be expanded with detailed descriptions of both the methods used and the findings for various cancers and should be updated to include followup into the 1980's. Recommendations for additional studies of the role, if any, of smokeless tobacco in the etiology of cancers outside of the upper aerodigestive tract should await the results of these analyses.

On the basis of current knowledge, it can be assumed that chewing tobacco and snuff contain several unknown nitroso compounds that may be contributors to the carcinogenic potential of these products. In-depth analytical studies are needed for the identification of these unknown compounds. Furthermore, mechanisms of their *in vitro* and endogenous formation should be studied together with those of the nitroso compounds that are already known to occur in smokeless tobaccos. For the validation of the uptake of the major carcinogens by tobacco chewers and snuff dippers, markers should be measured in the target tissues and in physiological fluids. Major emphasis should be placed on the identification and assays of DNA-adducts with tobacco-specific compounds in tissues of the oral cavity.

Finally, trends over time in age-specific oral cancer incidence and mortality rates should be monitored to determine whether the increasing use of smokeless tobacco by Americans is influencing national or regional cancer patterns. Changes in the prevalence of use and in the characteristics of smokeless tobacco products should also be documented. Such monitoring will provide a base upon which future investigations of associations between smokeless tobacco and cancer can be built.

Chapter 3.

NONCANCEROUS AND PRECANCEROUS ORAL HEALTH EFFECTS ASSOCIATED WITH SMOKELESS TOBACCO USE

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INTRODUCTION

This chapter addresses the health effects of smokeless tobacco use on the oral tissues through a systematic review of the relevant scientific literature of animal and human studies. The major areas addressed are the effects of smokeless tobacco use on the oral soft tissues, the periodontium, and the teeth. This chapter also reviews information regarding the potential of oral tissue altered by smokeless tobacco use to transform to dysplasia and malignancy.

Within each area, except for the section on the transformation of oral soft tissues, those tissues or conditions that are suspected to be most affected by smokeless tobacco use, or that hold the greatest potential for health effects, are considered initially. Where contradictory evidence exists, these data are also presented. Studies that were judged to meet stringent selection criteria* are presented first, followed by data from less rigorous study designs and case reports.

Within the section on the transformation of oral soft tissues, the presentation of the evidence is grouped according to clinical reports, cohort studies, and case-control studies. This was done so as to be consistent with the format used in the chapter on Carcinogenesis Associated With Smokeless Tobacco Use (chapter 2). In some cases, studies referenced in this chapter are the same as those used in chapter 2. The reader should review both chapters to obtain all pertinent information contained in these studies.

Only studies from the United States and Scandinavia are included for the sections on oral leukoplakia/mucosal pathology, gingival and periodontal tissues, and salivary glands. This assures that studies dealing with similar types of smokeless tobacco are used for comparison purposes. However, the section on the transformation of oral soft tissues includes a fuller range of studies that have reviewed the histopathologic changes associated with smokeless tobacco-induced lesions. Studies investigating the histopathologic transformation of nonsmokeless tobacco-induced lesions have not been included.

A summary of selected studies that addresses study sample, methods, and observations is provided in table 1 as a ready alphabetical reference to the text. In addition, a summary of selected case reports is provided in table 2. Emphasis has been placed on the issues of prevalence of oral tissue changes, types of changes, site-specificity of changes, and the effects of dose-response.

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* See Introduction, Overview, and Conclusions for discussion of criteria for causality.

TABLE 1.—Selected Study Summaries for the Noncancerous Oral Health Effects From the Use of Smokeless Tobacco

Study	Sample	Methods	Observations	Comments
Axéll, 1976			**Leukoplakia/ Mucosal Pathology**	
	• 20,333 Swedes: 51% females, 49% males. • Ages 15 years and older.	• Cross-sectional design. • Data collected on tobacco habits, medications taken, oral hygiene status, and prosthetic status. • Clinical examinations utilized diagnosis based on specific clinical criteria. • Photographic documentation of all lesions diagnosed as leukoplakia or lichen planus. • Tissue specimens taken of selected cases. • Statistical analysis conducted: t-tests, chi square tests, and, if appropriate, Fisher's exact test.	• Of 1,444 snuff users, 116 (8%) had "snuff dipper's lesion" (oral leukoplakia). • The prevalence of oral leukoplakia was 3.6% among the total population examined.	• It is not clear how many of the snuff users were also tobacco smokers. • Snuff dipper's lesion implies mucosal tissue changes at the site of snuff placement.
Greer and Poulson, 1983			**Leukoplakia/ Mucosal Pathology**	
	• 1,119 teenagers in grades 9-12. • 117 (10.5%) smokeless tobacco users: 113 males, 4 females. • Denver, Colorado.	• Cross-sectional design. • Questionnaire administered to determine years of use, frequency of use, brand of tobacco used, site of application, use of other confounding agents, and dental care history.	• A suggested association between level and duration of smokeless tobacco use and mucosal lesions (42.7% of smokeless tobacco users had oral mucosal lesions).	• An analysis of the influence of cofactors was not conducted. • No statistical analyses reported. • Examiners blind to responses on questionnaire. • No comparisons reported between users of smokeless tobacco and nonusers.

TABLE 1.—Continued

Study	Sample	Methods	Observations	Comments
Greer and Poulson, 1983 (cont.)			**Gingival and Periodontal**	
		• Clinical examination conducted of soft and hard oral tissues. • Lesions graded according to a scale developed by Axéll et al. (1976) and modified by Greer and Poulson.	• 26% of smokeless tobacco users had site-specific gingival recession. • Users with lesions had longer use and higher daily exposure than users without lesions.	• Smokeless tobacco-associated periodontal degeneration defined. • Did not assess the interrelationship of smokeless tobacco, cigarettes, and alcohol.
			Teeth	
			• " found no evidence of tobacco-associated dental caries." • No evidence of occlusal or incisal abrasion. • One case of cervical erosion.	
Greer et al., 1986			**Salivary Glands**	
	• 45 smokeless tobacco users (43 males and 2 females); 15 subjects in each group known as juveniles, young adults, and geriatric. • Ages 13-74 years. • Denver, Colorado.	• Cross-sectional design. • Lesions graded by classification developed by Greer and Poulson, 1983. • Examined only lesions classified according to scheme. • Histomorphological methods used on tissue specimens. • No statistical analysis conducted.	• Of 18 tissue samples with salivary glands, 4 demonstrated sialadenitis and degenerative changes. • A routine pattern of chronic sialadenitis was not shown for any of the three age groups. • Four patients (ages 21, 25, 50 and 66) showed either mild, moderate, or severe salivary gland fibrosis.	• Authors suggest that the degree of salivary gland fibrosis, degenerative change, and sialadenitis may be associated with tobacco brand instead of a generalized response caused by all tobacco.

TABLE 1.—Continued

Study	Sample	Methods	Observations	Comments
Hirsch et al., 1982			**Leukoplakia/ Mucosal Pathology**	
	• 50 male habitual snuff dippers. • 41.3-year mean age (range 15-84 years). • Sweden.	• Cross-sectional design. • Subjects classified on a four-degree scale of lesion severity (developed by Axéll et al., 1976); biopsies were taken. • Histomorphological and histochemical methods conducted on subjects' tissue specimens. • Tobacco and alcohol use histories ascertained from a questionnaire.	• Interpretation of histomorphological and histochemical results demonstrated that the oral mucosal reaction to snuff-induced hyperplasia in the basal cell layers. • Lethal damage was found in surface layers. • Duration of use and daily exposure to smokeless tobacco were shown to affect the severity of the leukoplakia. • Dysplasia could not be predicted by using suggested clinical degree of lesion classification.	• Dose considerations were made and confounding variables considered. • Differences in brand of tobacco used were taken into account.
			Salivary Glands	
	• Tissue specimens from 74% of patients included salivary glands.	• Statistical analysis conducted: one-way analysis of variance and multiple comparisons using the Scheffe method.	• The salivary glands and excretory ducts showed degenerative changes of a more severe nature than found in the surface epithelium. • 42% of salivary glands demonstrated sialadenitis and degenerative changes. • Weak oxidative enzyme activities noted in acinic cells in salivary glands with sialadenitis and degenerative changes. • Some signs of metabolic atypia noted. • Markedly degenerative changes seen in salivary glands associated with the more severely, clinically classified lesions.	• Degenerative changes not specifically defined by authors. • Authors state that variations in degenerative changes of salivary glands may be because of differences in brands of snuff and snuff-dipping habits.

TABLE 1.—Continued

Study	Sample	Methods	Observations	Comments
Jungell and Malmström, 1985	• 441 military recruits. • Ages 17-19 years. • Finland. • 48 (11%) were snuff users. • 18.9-year mean age (range 17-21 years).	• Cross-sectional design. • Questionnaire administered to ascertain tobacco product use and drinking habits and frequency of dental care. • Clinical examination conducted. • Biopsies taken of 21 snuff users with lesions. • Resting and stimulated (paraffin served as the stimulator) salivary excretions measured. • Statistical analysis conducted: t-test. • 10 nonusers of snuff also measured for salivary excretions.	**Salivary Glands** • Resting salivary flow of snuff users was significantly higher than that of nonusers. • Stimulated salivary flow was higher, but not significantly, among snuff users than among controls. • There was no difference in buffering capacity between the two groups.	• Authors interpret difference in resting salivary flow to be a reaction to the presence of the local irritant snuff.
Modéer et al., 1980	• 232 school children: 119 males, 113 females. • 13.5 years mean age. • 11% of males were regular snuff users. • Sweden.	• Cross-sectional design. • Interviewed about tobacco product use history and oral hygiene practices. • Standardized dental indices used to measure changes in oral hygiene and periodontal conditions. • Dental caries assessed clinically and radiographically. • Statistical analyses conducted: cross tabulations, multiple regression, and student's t-test.	**Gingival and Periodontal** • The use of snuff demonstrated a significant relation to gingivitis after controlling for plaque. • Effects of snuff on the gingival tissue included both location of the snuff and as a predictor of gingivitis in general.	• Authors state snuff use may influence gingival tissue directly resulting in gingivitis. • Examiners blind to responses from interview.

TABLE 1.—Continued

Study	Sample	Methods	Observations	Comments
Offenbacher and Weathers, 1985			**Leukoplakia/ Mucosal Pathology**	
	• 565 males from 5 schools. • 13.8-year mean age (range 10-17 years). • 75 (13.3%) smokeless tobacco users. • Georgia.	• Cross-sectional design. • Questionnaire used to obtain history of tobacco product use, dental visits, and social history. • Intraoral examination conducted using some standardized indices. • Statistical analyses included: chi square, odds ratios, kappa coefficient calculations, and t-tests. • Control group used.	• Frequency of occurrence of soft tissue pathology was significantly elevated in users (primarily due to increased prevalence of white mucosal lesions). • No attributable risk for mucosal pathology in smokeless tobacco users who were free of gingivitis.	• Soft tissue indices are not described. • Method of selecting schools for subject ascertainment not described. • Confounding variables considered.
			Gingival and Periodontal • No relationship between smokeless tobacco use and the prevalence of gingivitis. • Prevalence of gingival recession significantly elevated in smokeless tobacco users. • A significant attributable risk exists for gingival recession in smokeless tobacco users.	• Smokeless tobacco use is viewed as a cofactor with the presence of gingivitis in promoting gingival recession. • No clinical definitions provided for the assessment of gingivitis or gingival recession.
			Teeth • Smokeless tobacco users with gingivitis had significantly greater caries prevalence compared with nonusers without gingivitis. • Prevalence of caries was significantly greater in users with gingivitis who used both snuff and chewing tobacco compared with nonusers with gingivitis or those who were gingivitis free.	

TABLE 1.—Continued

Study	Sample	Methods	Observations	Comments
Peacock et al., 1960			**Leukoplakia/ Mucosal Pathology**	
	• 1,338 employees of local textile mill. • North Carolina.	• Cross-sectional design. • Interviewed about tobacco product use and given an oral examination.	• Highly significant relationships between chronic snuff and tobacco use and oral leukoplakia development found for all age groups and for both sexes.	• Examiners blind to interview responses. • 90% of employees had either poorly fitting complete dentures or only few and carious teeth. • Many employees have had the habit since they were 3 years old.
Poulson et al., 1984			**Leukoplakia/ Mucosal Pathology**	
	• 445 subjects: 52% females, 47% males. • 56 (12.6%) smokeless tobacco users (all males). • 16.7-year mean age (range 14-19 years). • Rural Colorado.	• Cross-sectional design. • Questionnaire administered (same as one used in Greer and Poulson, 1983). • Clinical examination conducted of oral hard and soft tissues. • Lesions graded by classification developed by Greer and Poulson, 1983.	• Of 56 smokeless tobacco users, 35 (63%) had lesions of the hard or soft tissues. • 33 (58.9%) smokeless tobacco users had mucosal alterations. • Mucosal lesions were found in area of quid placement. • Duration of use and length of daily exposure were factors in the development of lesions. • Multiple lesions in the same subject reported.	• Examiners blind to responses on questionnaire. • Definitions of clinical states provided. • Comparisons to nonusers not reported. • A history of confounding variables obtained. Effects of variables not addressed statistically.
			Gingival and Periodontal	
			• Of 56 smokeless tobacco users, 15 (27%) had site-specific gingival recession: 2 users had periodontal lesions only; 13 had both mucosal lesions and periodontal destruction.	• Periodontal degeneration defined. • Effects of confounding variables not addressed statistically.

TABLE 2.—Summary of Selected Case Reports

Study	Country	Number of Users	Age	Product Used	Duration of Use	Findings
Archard and Tarpley, 1972	USA	3	31 42 60	Snuff Snuff Snuff	11 years 20 years 50 years	A homogeneous eosinophilic submucosal deposit above the minor salivary glands did not initiate an inflammatory response nor support the possibility that the deposits were amyloid.
Christen, Armstrong, and McDaniel, 1979	USA	1	36	Snuff	13 years	Gingival recession, clinical leukoplakia, periodontal bone loss, and tooth abrasion found where tobacco was habitually placed.
Christen, McDaniel, and Doran, 1979	USA	14	18-22	Snuff, chewing tobacco	6 months to 9 years	8/14 with clinically detectable gingival recession; 9/14 with clinical leukoplakia; 11/14 with erythematous soft tissue changes where tobacco or snuff was habitually held.
Frithiof et al., 1983	Sweden	21	31-79	Snuff	10-60 years	21/21 with snuff-induced lesions localized to area where snuff was held; 2/21 with observable gingival retraction.
Hoge and Kirkham, 1983	USA	1	20	Snuff	1 year	Gingival recession and hyperkeratosis found where tobacco was habitually placed.
Pindborg and Poulson, 1962	Denmark	7	Not reported	Snuff	20-30 years	4/7 had whitish mucous membrane with a delicately folded appearance at site of snuff placement.
Pindborg and Renstrup, 1963	Denmark	12	39-83	Snuff	20-50 years	12/12 with mucous membrane that was "whitish, sometimes yellowish-brown, dry appearance with a very delicately folded or finely grooved surface."
Zitterbart, Marlin, and Christen, 1983	USA	1	36	Chewing tobacco	24 years	Gingival recession, "smokeless tobacco-users lesion," and abraded occlusal surfaces of posterior teeth found where tobacco was habitually placed.

THE EFFECTS OF SMOKELESS TOBACCO USE ON ORAL LEUKOPLAKIA/MUCOSAL PATHOLOGY AND THE TRANSFORMATION OF ORAL SOFT TISSUES

Oral Leukoplakia/Mucosal Pathology

Background and Definitions

Various oral soft tissue effects of smokeless tobacco use have been reported in the literature. These effects include oral leukoplakia/mucosal pathology. The actual terms used and the definitions employed to describe these conditions vary widely from study to study (table 3). The World Health Organization (WHO) defines oral leukoplakia as a white patch or plaque that cannot be characterized clinically or pathologically as any other disease *(1)*. The mucosal pathology that is found in smokeless tobacco users also has been referred to as hyperkeratosis, an oral mucosal lesion that exhibits an abnormal whitish (keratinized) appearance clinically. The authors' terms are employed when a specific study's findings are described. However, in the discussion portion of the report, the general terms of oral leukoplakia/mucosal pathology are used.

The association between smokeless tobacco use and oral leukoplakia/mucosal pathology has been moderately studied. The WHO has stated that tobacco is an etiologic agent for the formation of oral leukoplakia *(1)*. This association was reaffirmed at an International Seminar on Oral Leukoplakia and Associated Lesions Related to Tobacco Habits *(2)*. In a review of the effects of tobacco habits other than smoking, the use of smokeless tobacco/snuff was associated with the presence of leukoplakia *(3)*.

Studies in the United States

Six studies have addressed the prevalence of oral leukoplakia/mucosal pathology in smokeless tobacco/snuff users *(4-9)*. In two of these studies, blindness of the examiners toward the tobacco habits of the subjects was maintained, and oral tissue findings in smokeless tobacco users and nonusers were compared *(7,9)*. Three of these studies investigated adults *(4-6)* and three investigated adolescents *(7,9)*. In addition, several case reports have described oral leukoplakia/mucosal pathology findings in smokeless tobacco users *(10-13)*. Highlights of these studies and reports are summarized below.

Offenbacher and Weathers investigated the oral tissue effects of smokeless tobacco use in adolescent males from the greater metropolitan area of Atlanta, Georgia *(9)*. They used oral examinations and self-administered questionnaires on tobacco use. Of the 565 males who were examined, 75 (13.3 percent) used smokeless tobacco. The difference in the prevalence of mucosal pathology in smokeless tobacco users (22.7 percent) was statistically significant compared with that of nonusers (4.7 percent); however, the authors did not provide specific diagnostic

TABLE 3.—Variations in Terms Used and Definitions Provided for Leukoplakia/Mucosal Pathology Associated With Smokeless Tobacco Use by Studies Cited

Study	Term(s) Used	Definition(s) Provided	Comments
Axéll, 1976	Snuff-dipper's lesion.	A four-category classification scheme based on tissue color, wrinkling, and thickening was used.	The authors believe that this is a well-defined irritation that excludes it from the diagnosis of leukoplakia.
Christen, Armstrong, and McDaniel, 1979	Clinical leukoplakia.	"Implies only the clinical feature of a white patch or plaque on the oral mucosa which will not rub off and which cannot be characterized clinically or histologically as any other specific disease."	The authors cite the WHO 1978 and Waldron and Shafer 1975 references *(1,47)*.
Christen, McDaniel, and Doran, 1979	Leukoplakia.	"Implies only the clinical feature of a white plaque on the mucosa . . ."	The authors cite the Waldron and Shafer 1960 reference *(48)*.
Frithiof et al., 1983	Snuff-induced lesion.	"Tissue changes in the oral mucosa" that are due to snuff use.	The authors cite the WHO 1978 reference for the definition of leukoplakia and state that "since the snuff-induced lesion, with its typical clinical pattern and its specific etiology, obviously constitutes a definite diagnostic entity, the term 'leukoplakia' is avoided . . ."
Greer and Poulson, 1983	Oral mucosal lesions (alterations) associated with the use of smokeless tobacco.	These lesions were defined by a modification of a clinical grading method developed by Axéll et al., 1976.	In addition, lesions were classified by their texture, contour, and color.
Hirsch, Heyden, and Thilander, 1982	Snuff-induced lesions.	These lesions were defined by the grading method developed by Axéll et al., 1976.	—

TABLE 3.—Continued

Study	Term(s) Used	Definition(s) Provided	Comments
Hoge and Kirkham, 1983	Hyperkeratotic-appearing tissue.	No definition is provided, although the authors discuss the "formation of a hyperkeratotic zone in the region of the 'snuff pouch' where the tobacco is habitually held."	The authors cite the Shafer, Hine, and Levy 1969 reference *(49)*.
Moore, Bissinger, and Proehl, 1952	Oral leukoplakia.	No definition provided.	—
Offenbacher and Weathers, 1985	Mucosal pathology, soft tissue pathology.	No definitions provided.	The pathological findings identified by the investigators included morsicatio, ulcer, keratosis/leukoplakia, vesiculobullous, petechiae, abscess, erythema, mucocele, and pericoronitis.
Peacock, Greenberg, and Brawley, 1960	Leukoplakia.	"A pearly white plaque on the mucous membrane which could not be scraped off with a tongue blade."	—
Pindborg and Poulson, 1962	Leukoplakia.	No definition provided.	The investigators described the mucous membrane as having a slightly whitish, delicately folded appearance.
Pindborg and Renstrup, 1963	Snuff-induced leukoplakia.	No definition provided.	The investigators described the leukoplakias as "slightly whitish, sometimes yellowish-brown, dry appearance with a very delicately folded or finely grooved surface."
Poulson, Lindenmuth, and Greer, 1984	Oral mucosal lesions (alterations) associated with the use of smokeless tobacco.	The clinical appearance of these lesions was defined by a grading method developed by Greer and Poulson, 1983.	Alterations in texture, color, and contour of the mucosal lesions also were identified.
Zitterbart, Marlin, and Christen, 1983	Generalized smokeless tobacco-users lesion.	No definition provided.	The lesion was described clinically as "peculiarly wrinkled and thickened."

criteria in this assessment. The range of mucosal pathologic findings included such conditions as morsicatio (cheek biter's lesion), ulcer, keratosis/leukoplakia, vesiculobullous, petechiae, abscess, erythema, mucocele, and pericoronitis. Although 50 percent of the smokeless tobacco users with mucosal pathology had keratosis/leukoplakia compared with 3.8 percent of the nonusers with mucosal pathology, the authors did not identify the locations of the mucosal pathologies.

Peacock, Greenberg, and Brawley reported a significant relationship between chronic tobacco use and the presence of oral leukoplakia[*] in a study of 1,388 textile mill workers in North Carolina *(5)*. The 362 employees who reported using smokeless tobacco had a significantly higher prevalence of leukoplakia (34 percent) than did the 457 nonusers (7.4 percent). In addition, the authors noted a direct leukoplakia and age effect.

In a study conducted in Denver, Colorado, Greer and Poulson examined 1,119 teenagers in grades 9 to 12 to assess the relationship between oral tissue alterations and the use of smokeless tobacco *(7)*. Smokeless tobacco was used by 117 (10.5 percent) of these teenagers. Of these, 42.7 percent had oral mucosal lesions[†] in the area of tobacco placement. Forty-six percent of the teenagers with mucosal lesions also had concomitant periodontal tissue degeneration.[‡]

Poulson, Lindenmuth, and Greer examined a sample of 445 teenagers in five rural Colorado towns to assess the relationship betwen oral tissue alterations and smokeless tobacco use *(8)*. Smokeless tobacco was used by 56 (12.6 percent) of the teenagers. Of these, 58.9 percent had oral mucosal lesions in the area of habitual tobacco placement. Concomitant periodontal degeneration was noted in 39.4 percent of those with oral mucosal lesions.

Contrasting the results of rural versus urban adolescent smokeless tobacco users, Poulson, Lindenmuth, and Greer suggested that the duration of use may be critical in the development of "oral lesions" *(8)*.[§] Those adolescents with oral lesions used smokeless tobacco longer (an average of 3.3 years in the rural and urban groups) than those without lesions in both the rural and urban groups (2.3 years and 2.2 years, respectively). In addition, the authors noted similar effects of different levels of smokeless tobacco use in daily exposure. Users with oral lesions were exposed 205 minutes per day in the rural group and 177 minutes per day in the urban group compared with users with no oral lesions (110 minutes and 53 minutes, respectively). Also, more than twice as many marked oral mucosal lesions were identified in the rural population as in the urban population.

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[*] Leukoplakia was defined as a "pearly white plaque on the mucous membrane which could not be scraped off with a tongue blade."

[†] The authors used a modification of the classification method that was developed by Axéll et al. that identifies the oral mucosal lesions according to color, wrinkling, and thickening *(14)*.

[‡] The authors define this degeneration as "site-specific gingival recession with apical migration of the gingiva to or beyond the cementoenamel junction, with or without clinical evidence of inflammation."

[§] The term "oral lesions" used here includes periodontal tissue degeneration and oral mucosal lesions.

Smith et al. examined a population of 15,500 snuff users by cytological, histological, and visual means *(6)*. Of these users, 1,751 (11.3 percent) demonstrated oral mucous membrane changes. Although no definitions were provided, these changes were described as "cloudy or gray glistening" areas having "wrinkled appearance(s)" and presenting "white or red granular appearance(s)." The authors reported that when snuff was withdrawn, the tissue returned to normal appearance.

Moore, Bissinger, and Proehl investigated the relationship between tobacco use and oral cancer in male patients age 50 years and older who attended the General Tumor Clinic in Minneapolis, Minnesota *(4)*. The authors noted that a significant number of the patients who manifested oral leukoplakia (18 of 23—78.3 percent) used smokeless tobacco. A tobacco user in this study was defined as a person who used the tobacco product for 20 or more years. Apparently, some of these 23 patients were also pipe, cigar, or cigarette smokers, although the exact number was not specified. The authors indicated that the most severe patches of leukoplakia were seen in patients who chewed "strong" tobacco and over a longer duration (no quantification reported). In most instances in which patients had stopped using smokeless tobacco, leukoplakia disappeared.

Several case reports (table 2) have described oral leukoplakia/mucosal pathology at the site of smokeless tobacco/snuff placement *(10-13)*. These cases represent males of various ages with differing years of smokeless tobacco/snuff use. Hoge and Kirkham reported that in one patient, withdrawal of snuff resulted in a reversal of the hyperkeratotic lesions *(12)*.

Studies in Scandinavia

Studies of smokeless tobacco from Scandinavia have investigated the prevalence of oral leukoplakia/mucosal pathology in users *(15-19)*.

Axéll found 1,444 smokeless tobacco users (predominantly men) in the 20,333 Swedes who were examined for soft tissue lesions *(17)*. Of these users, 116 (8 percent) had "snuff-dipper's lesion" (see table 3 for definitions). The prevalence of oral leukoplakia among the total study population was 3.6 percent.

Hirsch, Heyden, and Thilander *(18)* graded oral mucosal lesions on an established four-point scale *(14)* and correlated these findings with the snuff habits in 50 Swedes ages 15 to 84 years who used snuff routinely. Younger patients were found to have lower degrees of pathologic changes, while a significant predominance of older patients was noted with higher degrees. The authors reported that patients with oral mucosal lesions of the highest degree had used snuff an average of 34.7 years compared with the 9.2- to 13.6-year average for patients with lower degrees of pathologic changes. They also noted that patients with high degrees of pathologic changes dipped twice as long per day (an average of 10.1 and 10.6 hours per day) as patients with lower degrees of pathologic changes (5.2 and 6.5 hours per day, respectively). Although these patients reported multiple tobacco habits, the authors stated that no differences in clinical grading were found between patients who used snuff only and those who used snuff and other tobacco products.

In addition, several case reports have described oral leukoplakia/ mucosal pathology (table 2). In Sweden, Frithiof et al. examined 21 male snuff users ages 31 to 79 years *(19)*. All had snuff-induced lesions that were localized to the area in the oral cavity where the tobacco was held. Similarly, leukoplakia lesions were found at the site of snuff placement in all 12 male users of snuff ages 39 to 83 years in a study in Denmark *(15)*. In this latter study, 3 weeks after one of the patients discontinued snuff use, the clinical appearance of the mucous membrane had returned to normal. In another report, four of seven Danish male users of snuff exhibited leukoplakia at the site of snuff placement *(16)*.

Discussion

The studies from the United States and Scandinavia demonstrate that oral leukoplakia/mucosal pathology is associated with smokeless tobacco/snuff use. In two studies, a higher prevalence of oral leukoplakia/mucosal pathology was found in users compared with nonusers of smokeless tobacco—22.7 percent compared with 4.7 percent *(9)* and 34.0 percent compared with 7.4 percent *(5)*. In all of these studies, between 8 and 59 percent of smokeless tobacco/snuff users were found to have oral leukoplakia/mucosal pathology.

It appears that the oral leukoplakia/mucosal pathology noted in smokeless tobacco/snuff users is found commonly at the habitual site of tobacco/snuff placement. Using a similar grading classification for snuff-induced lesions *(7,14)*, all of the mucosal pathology that was noted in four studies was at the site of habitual tobacco placement *(7,8,17,18)*. Similarly, the majority of the oral leukoplakia/mucosal pathology that was described in the case reports was found where the tobacco/snuff was usually placed.

The duration of use (in years) and daily exposure (in hours or minutes) to smokeless tobacco appear to be critical in the development and severity of oral leukoplakia/mucosal pathology. Three studies using similar approaches to the definition of oral leukoplakia/mucosal pathology and to the measurement of exposure noted this effect *(7,8,18)*.

Only two studies were designed to study the concomitant findings of oral leukoplakia/mucosal pathology and other tissue changes. The authors reported that 39.4 *(8)* and 46.0 *(7)* percent, respectively, of smokeless tobacco users with oral leukoplakia/mucosal pathology also had periodontal tissue degeneration (gingival recession). These oral soft tissue changes also were found at the site of habitual tobacco placement.

In several studies where individuals had stopped smokeless tobacco use, the oral leukoplakia/mucosal pathology disappeared *(4,6,12,15)*.

Transformation of Oral Soft Tissues

Background and Definitions

The previous section that discussed smokeless tobacco-induced leukoplakia noted that clinically observable changes in soft tissue morphology do occur as a result of smokeless tobacco use. Smokeless tobacco-associated lesions that have been traditionally classified as leukoplakias (white lesions) offer varying clinical degrees of differentiation and may persist or progress with continued smokeless tobacco use. Additionally, some leukoplakias have been observed to resolve clinically upon the cessation of smokeless tobacco use. This section of the report addresses the transformation of oral soft tissues. It discusses the potential for smokeless tobacco-induced lesions to regress, persist, or continue to progress to lesions with higher malignant potential or to malignancy.

There are varying clinical and histologic definitions in the scientific literature related to tobacco-induced changes (transformation) of oral soft tissues. The following definitions represent those most frequently encountered. It will be noted when significant variation of these definitions occurs in studies cited:

- Oral leukoplakia—a white patch or plaque that cannot be characterized clinically or pathologically as any other disease *(1)*.
- Snuff dipper's leukoplakia—a leukoplakia associated with the use of smokeless tobacco. These are further characterized as to differing morphologic forms.
- Erythroplakia—a lesion present as a bright red patch or plaque that cannot be characterized clinically or pathologically as any other condition, such as carcinoma or infection.
- Precancerous condition—a generalized state that is associated with an increased risk of cancer based on epidemiologic or histologic evidence.
- Precancerous lesion—a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart.
- Acanthosis—an increased thickness of the spinous cell layer of the epithelium.
- Hyperkeratosis—an increased thickness of the keratinized layer of the epithelium.
- Hyperparakeratosis—an increased thickness of a normally parakeratotic layer of the epithelium, i.e., surface cells with retained nuclei.
- Hyperorthokeratosis—an incrased thickness of a normally keratotic layer of the epithelium, i.e., surface cells without retained nuclei.

- Chevron keratinization—a keratinization pattern typified by vertical streaks of parakeratinization that extend to the epithelial surface and create surface irregularities by extensions of the outer surface layer.
- Dysplasia—abnormal tissue development characterized by varying numbers and degrees of morphologic cell changes that reflect grades of severity.
- Dysplastic changes include the following:
  - Pleomorphism in the size and shape of cells and their nuclei.
  - Abnormal numbers of cells undergoing mitotic activity (discrepancy in maturation).
  - Atypical mitotic cells.
  - Cytoplasmic atypicalities (altered nuclear to cytoplasmic ratio).
  - Hyperchromasia.
  - Irregular nuclear borders.
  - Basal cell hyperplasia.
  - Loss of polarity.
- Carcinoma in situ—a significant number of dysplastic epithelial cell changes that extend from the basal layer to the surface layer without violation of the basement membrane.
- Verrucous carcinoma—a clinically verruciform cancer of epithelial tissue that tends to be slowly and locally invasive with a metastasis and mortality potential that is lower than classic squamous cell carcinomas. The cells are well differentiated.
- Squamous cell carcinoma—a cancer of the stratified squamous epithelium that has varying clinical appearances, is invasive, extends beyond the basement membrane, and has a great potential for metastasis.

Evidence of the relationship between smokeless tobacco use and the transformation of oral soft tissues is represented by the following:

1. Clinical reports describing tobacco habits of persons with graded oral lesions.
2. Followup (cohort) studies of tissue changes, including transformation to malignancy, among patients with leukoplakia.
3. Case-control studies or case series of oral cancer describing concomitant leukoplakia.

A review of the evidence in each of these study areas follows:

Clinical Reports of Oral Lesions in Association With Smokeless Tobacco Use

Hirsch, Heyden, and Thilander *(18)* graded oral snuff-induced mucosal lesions in 50 patients on a four-point scale according to criteria developed by Axéll *(14)*:

- Degree 1: A superficial lesion with a color similar to the surrounding mucosa, slight wrinkling, and no obvious thickening.
- Degree 2: A superficial whitish or yellowish lesion with wrinkling and no obvious thickening.
- Degree 3: A whitish-yellowish to brown lesion with wrinkling, intervening furrows of normal mucosal color, and obvious thickening.
- Degree 4: A marked white-yellowish to brown lesion with heavy wrinkling, intervening deep and reddened furrows, and heavy thickening.

Snuff habits and drinking habits of the patients were obtained from questionnaires. Patients in the degree 4 category had been snuff dippers significantly longer than the rest of the patients. Also, patients in degrees 3 and 4 dipped approximately twice as long per day as did patients in degrees 1 and 2. The daily exposure to snuff was significantly longer in degree 4 (10.6 hours) than in degrees 1 (5.2 hours) and 2 (6.5 hours). When total exposure was compared between the four clinical groups taking into account hours of use per day as well as years of use, significant differences were found.

In this study, no significant differences could be found with regard to clinical grading and histological appearances between patients with multiple habits (snuff, smoking, and drinking) and those who only used snuff. The four clinical degrees of lesions exhibited an age-dependent effect with younger patients usually found in clinical degrees 1, 2, and 3 and a significant predominance of older patients noted in degree 4. Degree 4 lesions included an increased number of mitotic figures, edema, and slight to moderate inflammation compared with the other three degrees. Eighteen percent of the patients exhibited slight epithelial dysplasia, and lesions with slight epithelial dysplasia were found in all categories. Patients in the dysplastic group had been snuff dippers longer on average (23.9 years) as compared with those without dysplasia (19.5 years). No case of moderate or severe dysplasia was noted. (The authors referenced the WHO Collaborating Center for Oral Precancerous Lesions as the definition for dysplasia *(1)*.)

Axéll, Mörnstad, and Sundström obtained biopsies of the oral mucosal lesions of 114 male dippers ages 20 to 88 years from a sample of 1,200 Swedish snuff dippers *(14)*. Clinically, lesions were graded (degrees 1 through 4) based on color and morphology. Lesions of higher clinical degrees were associated with greater daily exposure to snuff in terms of hours and grams of exposure. All but one of the biopsies showed increased epithelial thickness. The outer layers appeared vacuolated with occasional remnants of cell nuclei. Lesions in degrees 3 and 4 had more pronounced surface layers. Acanthosis was evident in all of the clinical groups. None of the biopsies showed changes that were interpreted as cellular atypia or epithelial dysplasia. The cessation of snuff dipping for a few days was reported to result in clinical regression of the lesions with loss of the vacuolated layer.

Greer et al. reviewed clinically and histologically examined smokeless tobacco-induced leukoplakias from 45 patients ages 13 to 74 years *(20)*, following criteria that were previously established by Greer and Poulson *(7)* as adapted from Axéll. The vast majority of the mucosal lesions were corrugated, white, and raised. No evaluations for an interrelationship between smokeless tobacco use, smoking, and alcohol use and clinical or histologic tissue changes were attempted. Histologic examinations for specific changes were reported. Dark cell keratinocytes characterized by a strong affinity for basic dyes and by electron density of their cytoplasm and nucleus and suggested as dedifferentiated precursors of a neoplastic keratinocyte were found in 17 of 45 cases. However, their presence was unrelated to the clinical degree of the lesion. While they have also been observed in leukoplakias that are associated with smoking (or other causes), the control group of nontobacco-induced hyperkeratoses demonstrated dark cell keratinocytes in only 3 of 45 cases. Chevron keratinization of the epithelial layer representing altered cellular maturation was present in 42 of 45 smokeless tobacco-induced leukoplakias but in only 4 of 45 control leukoplakia cases. Koilocytotic changes appearing as vacuolated epithelial cells that may obscure the cytoplasm or appear with pyknotic nuclei, which are often associated with inclusion of viral particles in epithelial cells, were present in 27 of 45 smokeless tobacco-induced leukoplakias. In the entire sample of 45 cases, only 1 case of dysplasia (described as occurring in a long-term smokeless tobacco user) was identified. Three of the following characteristics had to be present for a lesion to be characterized as dysplastic:

- Loss of cellular polarity.
- Basal cell hyperplasia.
- Altered nuclear/cytoplasmic ratios.
- Anaplasia.
- Dyskeratosis.
- Atypical mitoses.

Because the dysplasia case also involved the use of alcohol and smoking, it is not possible to attribute its appearance solely to smokeless tobacco use.

In a study of 21 Finnish military recruits ages 17 to 21 years, mucosal lesions corresponded to the site of snuff placement and included the alveolar and labial mucosa to varying degrees *(21)*. The duration and intensity of snuff use for this specific group could not be determined from the study. Epithelial hyperplasia and acanthosis were universally found under the light microscope. Hyperorthokeratinization was noted in 12 cases, hyperparakeratinization in 9 cases, and Chevron-type keratinization in 1 case. One case of mild epithelial dysplasia was noted that included atypical and increased mitoses and loss of basal cell polarity. The authors concluded that this suggests a positive relation between snuff dipping and malignant changes.

Van Wyk biopsied 25 snuff-induced lesions from Bantu smokeless tobacco users whose lesions had existed from a few weeks to 40 years *(22)*. Comparison biopsies were also taken from healthy parts of the mucosa in the users, from healthy mucosa in nonusers, and from other white lesions and squamous carcinomas. From the biopsies obtained from snuff users, 18 cases of acanthosis, 23 cases of parakeratosis, 5 cases of keratosis, and 4 cases with numerous mitotic figures, pleomorphism, hyperchromatism, and an irregular basal cell layer were noted. Additionally, 11 showed a disrupted appearance of the basement membrane. Those not associated with inflammation were considered possibly to be premalignant. Epithelium featuring these characteristics has been referred to by some as "disquiet epithelium." Contrarily, the author stated that "the impression is gained that no relationship exists between oral malignancy and the use of snuff." This was based on the widespread use of snuff but the occurrence of only one case of alveolar or sulcular cancer (not in a snuff user) in the hospital during this study.

Several investigators have described connective tissue changes in snuff-induced lesions. A hyalinized, eosinophilic material that occurs well below the epithelium and around the minor salivary glands or in a plane that is generally parallel to the epithelial surface has been reported by Pindborg et al. *(16)*, Archard et al. *(23)*, Axéll et al. *(14)*, and Greer et al. *(20)*. The exact nature of and underlying explanation for the finding are not clear. Additionally, the role of such a histologic finding in the development or progression of premalignant or malignant lesions has not been identified.

Cohort Studies

Several investigations have followed persons with oral lesions for subsequent health outcomes. Smith reported the 10-year followup results on a group of patients with smokeless tobacco-induced leukoplakias *(24)*. In the original study, oral cytologies were performed on 1,751 patients presenting with leukoplakias out of 15,500 snuff users *(6)*. Results of the oral cytology examination consistently indicated only benign hyperkeratoses.* Biopsies were made of 157 leukoplakic lesions. However, no objective criteria for lesions selected for biopsy were offered. None of the biopsies showed changes consistent with dyskeratosis or malignancy. These patients were followed with repeat cytology smears for 5.5 years. No additional significant mucosal changes were reported. In a subsequent 4.5-year followup (10 years total followup), periodic biopsies were done on 128 of the 157 patients who had originally received biopsies *(24)*. The authors reported no dyskeratosis or carcinomas in the followup study. The method of followup was not specified. Significant numbers of patients were lost, and the clinical and histologic diagnostic criteria were not fully described.

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* The use of oral cytology for detecting dysplastic changes in leukoplakic lesions is less than satisfactory because of a high rate of false negative findings. The hyperkeratinized nature of leukoplakic lesions renders them resistant to the oral cytology scraping technique. Cellular changes in deeper layers of the epithelium would thus likely be missed *(25)*.

A prospective study of oral cancer among persons with oral leukoplakia or other possible precancerous lesions was conducted in the Ernakulum district, Kerala State, India, as part of a 10-year followup to a much larger study of 50,915 adults in 5 rural districts of India *(26)*. Among those individuals who had been diagnosed as having a leukoplakia during the original survey, there was a malignant transformation rate of 9.7/1,000 per year for those who only chewed tobacco. For those who both smoked and chewed, the rate was 5/1,000 per year, while no malignancies were reported for individuals with or without tobacco habits who had not had a previous oral lesion. The transformation rates among those with lesions were much higher than rates reported in the United States or European studies. While these results are not directly comparable to United States or European studies since the tobacco chewed in India is a variable mixture of betel leaf, areca nut, slake lime, and coarse tobacco, they suggest that the persons with leukoplakia are at increased risk of oral cancer. Specific clinical morphotypes of leukoplakia demonstrated varying potentials for malignant transformation: homogeneous, 2.27 percent; speckled, 21.4 percent; and ulcerated, zero percent.

In a small study of English coal miners, 8 of 22 patients with leukoplakia who chewed tobacco were followed for 5 years *(27)*. Five of the eight cases showed no advance in the lesions, and two showed regression. The author does not specify whether these were clinical or histologic determinations or whether the smokeless tobacco habit persisted in all cases. One lesion that had been regarded as benign showed some hyperorthokeratosis and acanthosis of the epithelium but with no more than "minor epithelial atypia." The clinical appearance of this lesion was reported to have regressed initially over an intermediate 2-year period despite continuance of the habit of tobacco chewing and smoking. Subsequent followup over a 2-year period indicated that the lesion had progressed to an exophytic squamous cell carcinoma. The site of the lesion was where the patient had held tobacco for 30 years. While the malignant transformation rate in the group of chewing tobacco-associated leukoplakias was 12.5 percent, the small numbers and high dropout rate limit the significance of the finding. Of significance was the unpredictable course of the malignant lesion, initially regressing and then transforming into a squamous cell carcinoma.

In a Danish study, 32 patients with snuff-induced leukoplakias from a group of 450 patients with leukoplakia were observed for a median time of 4.1 years *(28)*. Each patient had also used alcohol, with 17 percent claiming daily use. Thirty-three biopsies demonstrated hyperplastic epithelium with hyperparakeratosis in 87 percent of the cases; half showed vacuolated cells. One initial case of epithelial dysplasia was found, and one carcinoma was found to develop from a nondyskeratotic leukoplakia over the followup period. This represents a rate of premalignant or malignant transformation of 6.2 percent for either dysplasia or carcinoma. In comparing the rate of development of dysplasia and carcinoma from snuff-induced leukoplakias to nonsnuff-induced leukoplakias, the authors found no statistically significant differences. However, the rate of transformation in both groups was higher than would be expected in individuals without leukoplakic mucosa.

In an earlier report on a small sample of 12 white male snuff-using leukoplakia patients (use from 20 to 50 years), Pindborg and Renstrup did not find any malignant transformation *(15)*. Biopsies were taken from sites where the snuff was held. All 12 showed unkeratinized hyperplasia of the epithelium with a few deep streaks of parakeratosis and downgrowth and broadening of the rete pegs with the outer layers of cells being vacuolated and large. The authors state that snuff-induced leukoplakias are easily reversible. Based on the limited size of this sample, definitive conclusions could not be made.

Oral Lesions Concomitant With Oral Cancer

Three hundred and thirty-three patients with cancers of the buccal cavity and pharynx from the Robert Winship Memorial Clinic in Atlanta, Georgia, were compared with three control groups: a group with diseases of the mouth other than cancer or with no diseases; a group with cancer of sites other than the mouth, pharynx, or larynx; and a group without cancer and whose mouths were not examined—see chapter 2 *(29)*. The authors, citing leukoplakia as a precancerous condition, found leukoplakias "more commonly in women with low grade squamous carcinomas arising in the mouth and with multiple cancers. Snuff dipping was frequently associated with leukoplakia and low grade cancer arising in the mouth."

In a case-control study in Minnesota of cancers of the alveolar ridge, floor of the mouth, and buccal mucosa, it was noted that leukoplakias and cancers of the mouth were related to the use of snuff or chewing tobacco *(4)*. The most severe leukoplakias were reported among those who used "strong snuff" (no definition was provided) and held the quid at the same site for many years. Patients who quit using smokeless tobacco reportedly had leukoplakias disappear in most instances. A number of patients had multiple primary carcinomas that were also specific to the site of quid placement. Cancer lesions were described as having developed slowly over a period of several years, although no evidence of periodic clinical or histologic assessment was provided.

McGuirt reported on 76 oral cancer patients, most with carcinomas of the alveolar ridge or buccal mucosa, identified from the tumor registry at the North Carolina Baptist Hospital who had a documented history of heavy smokeless tobacco use *(30)*. Fifty-seven of these patients used snuff and reported no cigarette, pipe smoking, or alcohol habits. The range of use was from 10 to 75 years. Leukoplakias had previously been excised in 13.9 percent of the cases, and 47 percent had associated leukoplakias at the time of surgery. The author cited "panmucosal insult" from smokeless tobacco use as the cause of multiple lesions and recurrences—a type of field cancerization.

From histologic evaluations of oral tissue among 23 Swedish patients with anterior oral vestibular cancer who were snuff users, leukoplakic lesions were noted outside the snuff-associated tumor in 5 *(31)*. Leukoplakia and multiple carcinomas occurred together with the snuff-associated lesion in three cases. Eleven of nineteen cases assessed for presence of candida were positive. The temporal relationship between candida and carcinoma was not ascertainable, nor was the potential etiologic role of candida.

Rosenfeld and Callaway examined data from records at Vanderbilt University Hospital, Nashville General Hospital, and the office of Rosenfeld for cases of squamous cell carcinoma arising in the mucous membrane of the anterior two-thirds of the tongue, the floor of the mouth, the gingiva, and the buccal area *(32)*. A total of 525 cases were examined in users and nonusers of smokeless tobacco—300 occurred on the gingiva and buccal areas. Among women with cancer of the buccal or gingival area, 90 percent had a history of snuff use. While no periodic quantitative or qualitative assessment of the natural history of the cancers is provided, the authors do offer the following clinical impression of snuff-induced lesions in their study:

> These carcinomas arising in the inner cheek and gingiva frequently start as leukoplakia. Progressive thickening, cornification, and eventual cauliflower-like ulcerations ensue. All stages in the progressive disease may be seen in microscopic sections from a mere slight increase in the keratin layer, through carcinoma in situ to invasive malignancy.

Twenty-five cases of histologically confirmed buccal gingival cancer in female snuff users were identified at the University of Arkansas Medical Center from 1950 to 1959 *(33)*. Eleven cases occurred at buccal sites, 10 gingival, and 4 buccal and gingival. The patients (ages 44 to 84 years—mean 67.5) had a smokeless tobacco habit between 20 and 50 years. The lesions corresponded to the site of habitual tobacco placement. Leukoplakia was a concomitant lesion and had been present for many years. Repeat biopsies of lesions were made over long periods in some of the patients. Leukoplakic lesions from other parts of the mouth often showed atypia. An evolution from leukoplakia to pseudoepitheliomatous hyperplasia to early squamous cell carcinoma was found.

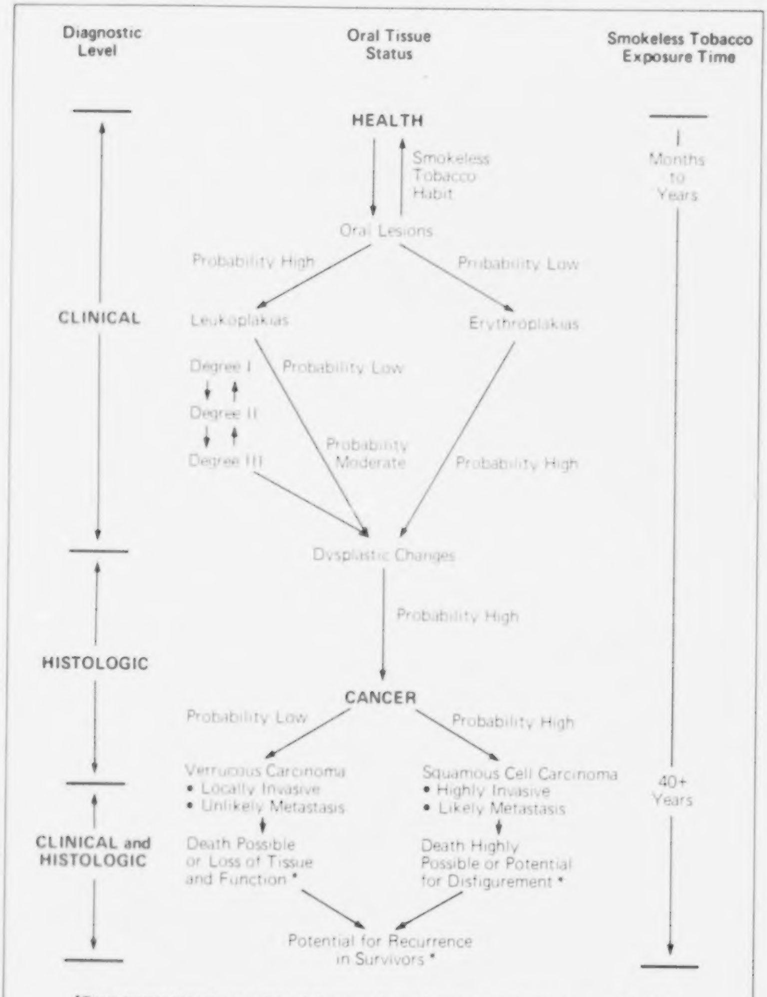
Discussion

In characterizing the role of smokeless tobacco use in the clinical and histologic course of oral lesions, there are several problems. First, oral leukoplakia should be considered a dynamic changing lesion of the oral mucosa *(34)*. Lesions retain the potential to resolve, remain static, or progress depending on a variety of factors that may be either exogenous (e.g., smokeless tobacco use) or endogenous (e.g., natural tissue defenses and repair potential). To achieve comparability of results among investigators, a standard system for gauging epithelial dysplasia is needed. Patients then could be followed prospectively to quantify the incidence of dysplastic change, incidence of transformation from a dysplastic state to a cancerous state, or in some cases transformation from an apparently benign to a cancerous state. But ethical considerations do not allow lesions to be monitored continuously from benign states to moderate and severe dysplasias and carcinoma in situ.

The next best alternative would be to provide estimates of risk for malignant transformation based on empirical and clinical observations or at least to quantify descriptively the association that smokeless tobacco-induced lesions have with other lesions or other potential etiologic factors. The body of literature on smokeless tobacco-induced lesions and their potential for malignant transformation allows for the development of a conceptual model of the natural history of smokeless tobacco-induced lesions (figure 1). This model is a composite of various prospective, retrospective, cross-sectional, and case studies that relate to smokeless tobacco-induced lesions. It depicts progressive changes that may occur in some individuals who are habitual users of smokeless tobacco and potential outcomes that could include death or disfigurement for some individuals who use smokeless tobacco for several decades. The data are clear that habitual smokeless tobacco use can produce mucosal lesions (see leukoplakia discussion). It is also clear that where groups of patients with smokeless tobacco-induced leukoplakias have been followed for several years, cases of cancer have been identified. Finally, when considering studies of oral cancers in habitual smokeless tobacco users, there appears to be a consistent finding of leukoplakias either having been previously excised in the area of habitual tobacco placement or being found concurrently with and in proximity to oral cancers.

In comparing studies on the transformation potential of smokeless tobacco-induced leukoplakias, it is found that different criteria have been used by various investigators in defining dysplastic changes. The number and nature of criteria that are considered and that are considered adequate to classify a case as dysplastic are not consistent. Additionally, the degree of agreement on diagnosis based on histology and clinical history between individuals has been shown to be quite variable. Pindborg, Reibel, and Holmstrup tested the degree to which a group of

FIGURE 1.—A Conceptual Natural History of Oral Mucosal Changes Associated With the Use of Smokeless Tobacco


Diagnostic Level
Oral Tissue Status
Smokeless Tobacco Exposure Time
HEALTH
Smokeless Tobacco Habit
Months to Years
Oral Lesions
Probability High
Probability Low
CLINICAL
Leukoplakias
Erythroplakias
Degree I
Probability Low
Degree II
Degree III
Probability Moderate
Probability High
Dysplastic Changes
Probability High
HISTOLOGIC
CANCER
Probability Low
Probability High
Verrucous Carcinoma
• Locally Invasive
• Unlikely Metastasis
Squamous Cell Carcinoma
• Highly Invasive
• Likely Metastasis
40+ Years
CLINICAL and HISTOLOGIC
Death Possible or Loss of Tissue and Function *
Death Highly Possible or Potential for Disfigurement *
Potential for Recurrence in Survivors *

*These factors depend upon stage of diagnosis, form of treatment and continuation of habit(s).

oral pathologists could agree on diagnoses where nine cases of epithelial dysplasia, carcinoma in situ, or initial squamous cell carcinoma were examined *(35)*. Color photomicrographs and information on the topography of the biopsy were presented. The authors' diagnoses were based on the criteria that are described in the report from the WHO International Collaborating Center for Oral Precancerous Lesions *(1)*. The degree of agreement with the authors' diagnoses for the nine cases ranged between 10 and 78 percent. This could partially explain the range in prevalence and incidence of malignant transformation that is reported by various investigators.

Other contributing factors in comparing studies could include different population groups in terms of age and gender and other confounding variables (e.g., smoking, alcohol use, and type of smokeless tobacco product used). Each of these limitations is suggestive of the type of research that is needed.

THE EFFECTS OF SMOKELESS TOBACCO USE ON THE GINGIVA, PERIODONTAL TISSUE, AND SALIVARY GLANDS

Background and Definitions

Reports of gingivitis, gingival recession, and degenerative salivary gland changes associated with smokeless tobacco use are contained in the literature. As with the previous section on oral leukoplakia, the terms used and the definitions employed to describe gingivitis and gingival recession vary widely from study to study. Table 4 displays the variations found in the literature. As each study is described in the following narrative, the authors' terms are employed. However, in the discussion portion of this report, the general terms of gingivitis and gingival recession are used. General definitions for these terms and for sialadenitis follow:

- Gingivitis—This condition refers to clinically detectable acute or chronic inflammation, either local or general, of the gingiva.
- Gingival recession—In general, this condition describes the apical migration of the gingiva with or without clinical evidence of inflammation.
- Sialadenitis—Inflammation of the salivary glands.

Gingival and Periodontal Tissue

Studies that assess the relationship between smokeless tobacco use and gingival and periodontal tissue effects are limited. The literature consists of several cross-sectional studies in teenagers and a few case reports.

TABLE 4.—Variations in Terms Used and Definitions Provided for Gingivitis and Gingival Recession by Studies Cited

Study	Term(s) Used	Definition(s) Provided	Comments
Christen, Armstrong, and McDaniel, 1979	Gingival recession, periodontal pocket, and loss of alveolar bone.	No definitions provided.	The tissue changes were described in general by the authors.
Christen, McDaniel, and Doran, 1979	Clinically detectable gingival recession.	No definitions provided.	—
Greer and Poulson, 1983	Tobacco-associated periodontal degeneration and periodontal lesions.	"Defined as site-specific *gingival recession* with apical migration of the gingiva to or beyond the cementoenamel junction, with or without clinical evidence of inflammation."	—
Hoge and Kirkham, 1983	Gingival recession.	No definition provided.	The authors defined the recession as having "exposed approximately 5 mm of labial root surface" and having destroyed the "entire functioning border of keratinized gingiva."
Modeer, Lavstedt, and Åhlund, 1980	Gingivitis/gingival inflammation.	Estimated on the basis of the gingival index of Löe and Silness, 1963 *(50)*.	—
Offenbacher and Weathers, 1985	Gingivitis.	No definition provided.	—
	Gingival recession.	No definition provided.	The gingival recession was "considered slight to moderate, ranging in 1-4 mm apical migration when present."
Poulson, Lindenmuth, and Greer, 1984	Tobacco-associated periodontal degeneration (other terms include "periodontal deterioration," and "localized periodontal degeneration associated with the site of tobacco placement").	"Defined as site-specific *gingival recession* with apical migration of the gingiva to or beyond the cementoenamel junction, with or without clinical evidence of inflammation."	—
Zitterbart, Marlin, and Christen, 1983	Gingivitis.	No definition provided.	—
	Gingival recession.	No definition provided.	The clinical findings were described for each tooth site involved.

Studies in the United States

Three cross-sectional studies have investigated the relationship of gingival and periodontal tissue changes and smokeless tobacco use in teenagers in the United States *(7-9)*. Offenbacher and Weathers examined the effects of smokeless tobacco use on mucosal pathology, on the presence of gingivitis and gingival recession, and on dental caries status (discussed in next section) *(9)*. Of the 75 smokeless tobacco users, the authors noted 72 percent with gingivitis and 60 percent with gingival recession. In those with gingival recession, 6.6 percent presented with recession in direct juxtaposition to the location of the tobacco placement. The authors did not describe how many users of smokeless tobacco had demonstrated combinations of these oral conditions. Also, no specific clinical definitions were given for the assessment of gingivitis or gingival recession, although the latter findings were described as "slight to moderate, ranging from 1 to 4 mm apical migration of gingival tissue." The higher prevalence of gingival recession among smokeless tobacco users (60 percent) as compared with that found in nonusers (14.1 percent) was found to be statistically significant. There were no statistically significant differences in gingivitis prevalence between smokeless tobacco users (72 percent) and nonusers (77.1 percent).

Of 117 adolescent smokeless tobacco users in Denver, Colorado, Greer and Poulson noted that 25.6 percent had tobacco-associated periodontal degeneration *(7)*. As noted earlier, this condition was defined as "site-specific gingival recession with apical migration of the gingiva to or beyond the cementoenamel junction, with or without clinical evidence of inflammation." Concomitant mucosal lesions were noted in 76.6 percent of those who had periodontal degeneration (gingival recession).

In a study of rural Colorado teenagers, Poulson, Lindenmuth, and Greer *(8)* described 26.8 percent of 56 smokeless tobacco users with periodontal degeneration (gingival recession) as defined by Greer and Poulson *(7)*. Eighty-seven percent of these had concomitant mucosal lesions.

Several case reports (table 2) describe the occurrence of gingival recession and periodontal tissue destruction in individual smokeless tobacco/snuff users *(10-13)*. The patients in these case reports were males who ranged in age from 18 to 36 years with varying duration of the smokeless tobacco/snuff habit ranging from 1 to 24 years. Although not universally found, gingival recession was usually noted, and the majority of patients presented with recession that was specific to the site where the tobacco/snuff was habitually placed.

Periodontal bone loss at the site of snuff placement was described in another patient who used snuff for 13 years *(10)*. In one patient, 3 weeks after cessation of snuff use, there was no regeneration of the lost gingival tissue, although, as noted earlier, the hyperkeratotic areas had disappeared *(12)*.

Studies in Sweden

Modeer, Lavstedt, and Ahlund studied the oral health effects of smoking and snuff use in 232 Swedish school children ages 13 to 14 years (119 boys and 113 girls) *(36)*. Thirteen (11 percent) of the boys used snuff. The children were interviewed regarding their tobacco and toothbrushing habits, and examiners (blind to the interview results) clinically assessed the degree of gingival inflammation, oral hygiene, and the presence of calculus (discussed in the next section). Standardized indices were used to assess all oral conditions. Controlling for the presence of dental plaque, gingival inflammation was the only variable that was significantly different between snuff users and nonusers. Snuff use was directly correlated with the degree of gingival inflammation. The gingival inflammation noted was related to the site of smokeless tobacco placement.

Discussion

The relationship of smokeless tobacco use and the health of gingival and periodontal tissue has received minimal study. Because of the variation in study designs and diagnostic criteria, comparisons between available studies are inappropriate. Thus the effects of smokeless tobacco use on these tissues are not clearly understood.

With regard to gingivitis, one cross-sectional study noted no difference between users and nonusers *(9)*. Another study, however, emphasized that there was a significant difference between users and nonusers and that snuff use was directly correlated with the degree of gingival inflammation *(36)*.

Gingival recession is a common finding among users of smokeless tobacco/snuff. In the U.S. cross-sectional studies, gingival recession was found in 25.6 to 60 percent of teenage users *(7-9)*. In the two Colorado studies, all the gingival recession was specific to the site of tobacco placement (25.6 and 26.8 percent) *(8)*. In the Georgia study, only 6.6 percent of the gingival recession was in the area of tobacco placement *(9)*. In addition, several case reports have identified gingival recession at the site of habitual tobacco placement *(10-13)*.

Between 76.6 and 86.6 percent of smokeless tobacco users who had gingival recession also had concomitant mucosal pathology *(7,8)*. These soft tissue changes were found at the site of habitual tobacco placement.

Salivary Glands

Smokeless tobacco or its components may contribute to degenerative changes and severe damage, such as undifferentiated carcinoma, to the salivary glands and excretory ducts of humans and mice *(18,20,28,37)*. In a study that assessed the formation of tobacco-specific nitrosamines from the major tobacco alkaloid nicotine, Hecht et al., reporting from the histologic evaluation, noted two undifferentiated carcinomas of the salivary glands in two groups of mice that were given injections of nitrosonornicotine (NNN) in saline or trioctanoin *(37)*. Because of the uncommonness of salivary tumors in strain A mice, Hecht et al. concluded that the tumors were probably a result of systemic administration of NNN.

Sialadenitis and degenerative changes in minor salivary glands were found in 16 of 50 habitual snuff dippers with a greater number belonging to the groups that were classified clinically as having the most severe snuff-induced lesions *(18)* (table 1). The findings from this study included a decrease in oxidative enzyme activities and indications of metabolic atypia that were based on enzyme histochemical tests. The salivary glands appeared to manifest more damage than the oral epithelium from snuff use. Variations in degrees of effect may be attributed to the variations in snuff dipping habits and brands of snuff.

In a recent study by Greer and his colleagues *(20)* (table 1), 45 smokeless tobacco users ages 13 to 74 years were clinically and histomorphologically assessed for the effects of smokeless tobacco on the oral tissues. Of 45 tissue specimens, 18 included salivary gland tissue. Damage in the form of sialadenitis and other degenerative changes in salivary glands was shown in 4 of the 18 specimens. A consistent pattern for chronic sialadenitis was not found among any of the age groups. The authors did not specify the other degenerative changes. However, four patients, ages 21, 25, 50, and 60 years, demonstrated either a mild, moderate, or severe salivary gland fibrosis. The most severe salivary gland fibrosis was found in the 21-year-old subject who was considered a short-term smokeless tobacco user; a definition for short-term user was not provided. Unlike the findings of Hirsch, Heyden, and Thilander *(18)*, salivary gland fibrosis or changes were not related to the stage (degree) of the clinical lesion. The authors concluded that there is no doubt that salivary gland fibrosis can be shown and that it is likely to be related to the damage from smokeless tobacco. They also commented that "It is likely that the degree of salivary gland fibrosis and degenerative change, along with sialadenitis, may be a factor that is associated with tobacco brand rather than with a generalized reaction caused by all tobacco."

Included among the many questions concerning the effects of smokeless tobacco use on the salivary glands is that of changes on the flow and buffering capacity of saliva. In a sample of 48 Finnish snuff users ages 17 to 21 years (mean 18.9), the resting and stimulated salivary flow was measured *(21)* (table 1). The subjects refrained from the use of snuff for 1 hour before collection of saliva. The saliva of 10 nonusers was similarly collected. The statistically significant findings demonstrated a higher resting salivary flow of snuff users compared with controls. Although the stimulated salivary flow was also higher among the snuff users than the controls, this difference was not statistically significant. Buffering capacity was the same between the two groups. Although these findings offer additional information regarding the effects of smokeless tobacco on the salivary glands, the clinical significance of these effects has not been systematically assessed, nor have the outcome differences related to the different products. Replication studies of these findings are needed before firm conclusions can be made.

In contrast to the effects just cited, Archard et al. were unable to identify lesions or dysfunctions associated with smokeless tobacco use *(23)* (table 2). These investigators carried out histochemical tests on lesions in the oral cavity that were in close proximity to the salivary glands. These tests revealed no evidence of an inflammatory reaction associated with the glands.

Discussion

The interpretation of data within this general area requires caution. Limited evidence suggests a possible relationship between the use of snuff and damage to the salivary glands. Should this be the case, the loss of salivary gland function can result in the decreased production of saliva and the ultimate loss of a protective buffer for the oral epithelium and the teeth against numerous exogenous factors such as infectious agents, including dental caries.

THE EFFECTS OF SMOKELESS TOBACCO USE ON TEETH

Background and Definitions

This section of the chapter addresses the role of various forms of smokeless tobacco in causing or contributing to diseases or conditions of the teeth. Specific effects that are examined include dental caries, abrasion, erosion, plaque and calculus buildup, and staining. For purposes of discussion, definitions are offered for a number of terms that are considered to represent commonly held concepts of diseases and conditions of the teeth as evidenced in the relevant scientific literature.

- Dental caries—Clinically detectable cavitation of the coronal or root surfaces of the tooth that is caused by acid demineralization of colonizing bacteria on tooth surfaces.
- Abrasion—Clinically evident wear of the coronal portion of teeth either generally or focally that appears excessive for a patient of a given age. This is a mechanical effect that is caused by the action of abrasive substances or objects during normal functioning or by oral habits.
- Erosion—Loss of tooth structure that is attributable to a chemical agent.
- Plaque—Bacterial-laden, proteinaceous material that is continually deposited in the oral cavity through the proliferation of bacterial types.

- Calculus—A concretion that forms on the coronal and exposed root surfaces of teeth through the calcification of bacterial plaques.
- Staining—An extrinsic stain deposit that results in discoloration on tooth surfaces.

Dental Caries

Evidence for the effects of smokeless tobacco use on the teeth is available from several cross-sectional studies (table 1), from a limited number of case reports (table 2), and from a limited number of related investigations of the potential for constituents of smokeless tobacco to serve as predisposing or etiologic factors in the development of dental caries.

As previously mentioned, Offenbacher and Weathers reported on the oral soft and hard tissue effects of smokeless tobacco use in a study population that comprised 565 males with a mean age of 13.8 years *(9)*. This population typifies the age group that is commonly described as "the cavity-prone years." Although caries rates expressed as decayed, missing, or filled teeth (DMFT) were higher for smokeless tobacco users without gingivitis than for nonusers without gingivitis, these differences were not statistically significant. However, when DMFT scores for smokeless tobacco users with gingivitis were compared with scores for nonusers without gingivitis, a significantly higher caries prevalence was found among users. Among students who used both snuff and chewing tobacco, the DMFT score was 6.56 + 0.71. This score is significantly elevated compared with scores of nonuser gingivitis-free students and the nonuser group that had gingivitis. There was a 2.4-fold increase in disease experience. In this study, the presence of gingivitis was presented as a cofactor with smokeless tobacco use in the increased prevalence of dental caries. This finding has not been reported elsewhere, and the biologic explanation is unclear.

The differences that were noted in caries rates could not be accounted for based upon differences in oral hygiene or the frequency of dental visits—two factors that could potentially affect DMFT scores. The examiners had no knowledge from the self-reported survey forms of the history of smokeless tobacco use among the group that was examined; thus, a degree of study "blindness" was attained. Absolute blindness in these types of surveys is difficult because it is likely that some evidence of smokeless tobacco use (e.g., tobacco residues, stain, odor, and soft tissue effects) is observable. No quantifiable dose-response effect for smokeless tobacco use and dental caries was reported in this study. Dental caries is highly age dependent, and no age adjustment was made in the statistical analysis.

A cross-sectional study by Greer and Poulson of 1,119 teenage smokeless tobacco users and nonusers from urban Colorado demonstrated neither "tobacco-associated dental caries" nor occlusal or incisal abrasion of the teeth *(7)*. This finding is not surprising because abrasive effects are cumulative and would likely require a number of years to become evident. The abrasion that has been reported in smokeless tobacco users has been in adults who have used smokeless tobacco products, generally leaf and plug forms of tobacco, for years *(10,13)*. The Greer and Poulson study reported a single case of cervical erosion on the mandibular central incisors.

Some case reports have implied a causative role for smokeless tobacco in the development of dental caries *(38,39)*, while others have postulated a potential protective effect from caries *(13,40)*. The presumed mode of protection would be through a greatly increased salivary flow that may provide a buffering action. Additionally, there is evidence that various forms of smokeless tobacco contain fluoride, from a few tenths to several parts per million, which may offer some cariostatic protection *(41)*. At the same time, various types of smokeless tobacco contain up to five different forms of caries-promoting sugars *(42)*. Two studies reported that constituents in smokeless tobacco products either cause a proliferation of caries-producing bacteria in vitro or, at the least, do not inhibit bacterial growth in vitro *(43,44)*. The fluoride and sugar contents of smokeless tobacco vary by product type *(41)*. This may explain the inconsistent and equivocal results obtained by different investigators. Variations in reported caries rates, if truly reflective of the larger population of smokeless tobacco users, may represent the clinical outcome of a number of antagonistic or synergistic factors that operate while smokeless tobacco is used.

Other Hard Tissue Effects

Plaque, calculus, and staining are extrinsic factors that may be associated with smokeless tobacco use. This is clinically important because dental plaque and calculus that is coated with plaque harbor bacteria that can produce acids and toxins and thus bring about dental caries and diseases of the periodontal structures. The staining of teeth, restorations, and prosthetic appliances have been described as resulting from smokeless tobacco use *(13,22,45,46)*. Van Wyk also reported a constant finding of chronic inflammation of tooth pulps that were extracted from oral snuff users *(22)*. He attributed this as being "probably due to the irritation of the snuff overlying the exposed dentine and cementum." No quantifiable evidence currently documents the risk of smokeless tobacco use compared with nonuse in the development of plaque, calculus, or staining or the relationship of staining to oral disease conditions.

CONCLUSIONS

1. Smokeless tobacco use is responsible for the development of a portion of oral leukoplakias in both teenage and adult users. The degree to which the use of smokeless tobacco affects the oral hard

and soft tissues is variable depending on the site of action, type of smokeless tobacco product used, frequency and duration of use, predisposing factors, cofactors (such as smoking or concomitant gingival disease), and other factors not yet determined.

2. Dose response effects have been noted by a number of investigators. Longer use of smokeless tobacco results in a higher prevalence of leukoplakic lesions. Oral leukoplakias are commonly found at the site of tobacco placement.
3. Some snuff-induced oral leukoplakic lesions have been noted upon continued smokeless tobacco use to undergo transformation to a dysplastic state. A portion of these dysplastic lesions can further develop into carcinomas of either a verrucous or squamous cell variety.
4. Recent studies of the effects of smokeless tobacco use on gingival and periodontal tissues have resulted in equivocal findings. While gingival recession is a common outcome from use, gingivitis may or may not occur. Because longitudinal data are not available, the role of smokeless tobacco in the development and progression of gingivitis or periodontitis has not been confirmed.
5. Evidence concerning the effects of smokeless tobacco use on the salivary glands is inconclusive.
6. Negative health effects on the teeth from smokeless tobacco use are suspected but unconfirmed. Present evidence, albeit sparse, suggests that the combination of smokeless tobacco use in individuals with existing gingivitis may increase the prevalence of dental caries compared with nonusers without concomitant gingivitis. Reports of tooth abrasion or staining have not been substantiated through controlled studies; only case reports are available.

RESEARCH NEEDS

The review of the literature for this component of the report has identified the need for research in each of the areas discussed: the oral soft tissues, the periodontium, the salivary glands, and the teeth. Basically, the effects of the various types and forms of smokeless tobacco in all age groups should be investigated. Controlled studies and comparisons between users and nonusers of smokeless tobacco are needed. Established criteria for assessing tissue changes and disease presence should be applied to permit comparability between studies.

Studies should include the identification and control of variables that also may affect these tissues. Such variables may include alcohol use, diet, oral hygiene practices, microbial flora changes, and salivary flow rate, composition, and pH. In addition to these variables, consideration should be given to the effects of concurrent disease states. For example, the effects of smokeless tobacco on dental caries in the presence or absence of gingivitis should be investigated.

The natural history of smokeless tobacco-induced lesions resulting from continued, intermittent, and discontinued smokeless tobacco use needs investigation. Histopathologic evaluations and clinical examinations to determine the natural history of oral leukoplakia/mucosal pathology and salivary gland pathology are desirable to understand completely the extent and severity of smokeless tobacco oral effects.

In general, incidence and prevalence studies should be implemented. Prospective study designs should be pursued to assess the temporal relationship between smokeless tobacco use and various health effects. In addition, dose-response studies are needed to assess dose in terms of both duration of use (in months and years) and daily exposure (in minutes and hours).

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Chapter 4.

NICOTINE EXPOSURE: PHARMACOKINETICS, ADDICTION, AND OTHER PHYSIOLOGIC EFFECTS

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INTRODUCTION

This chapter examines the consequences of exposure to nicotine from smokeless tobacco. It draws from the vast literature on the effects of nicotine delivered via smoking and intravenously and includes recent evidence of the effects of orally delivered nicotine.

The first section describes the pharmacokinetics of nicotine, including absorption, distribution, and elimination. The data presented indicate that nicotine is present in smokeless tobacco in significant amounts and that users attain blood levels of nicotine similar to those produced by cigarette smoking.

The second section reviews the established evidence that nicotine is an addictive and dependence-producing substance, having a number of important characteristics in common with prototypic addictive and dependence-producing substances, as well as substantial experimental evidence of its abuse liability and dependence potential. Given the nicotine content of smokeless tobacco, its ability to produce high and sustained blood levels of nicotine, and the well-established data implicating nicotine as an addictive substance, one may deduce that smokeless tobacco is capable of producing addiction in users. In addition, very recent studies provide direct confirmation that nicotine delivered orally from smokeless tobacco and nicotine chewing gum is addictive, producing abuse liability and dependence potential.

The final section of the chapter reviews the multisystem physiologic effects of nicotine and examines the evidence pertaining to the potential contributory role of nicotine in the causation of several diseases.

PHARMACOKINETICS OF NICOTINE

Levels of Nicotine in Smokeless Tobacco

Tobacco is a plant product, and therefore differences exist in nicotine content among and within different strains of tobacco. Nicotine content among smokeless tobacco products also differs: moist snuff contains 4.56 to 15.1 mg nicotine per gram *(1)*; plug tobacco has been measured to contain 17.2 mg per gram *(2)*. Assuming a daily consumption of 10 grams of smokeless tobacco, the habitual user can be exposed to roughly 130 to 250 mg nicotine per day, of which varying amounts may be absorbed. By comparison, cigarette tobacco averages 15 mg nicotine per gram or 9 mg nicotine per cigarette *(3)*. A person who smokes a pack of cigarettes per day therefore can be exposed to 180 mg nicotine per day.

Absorption of Nicotine

Nicotine is a weak base (pKa 7.9). In its ionized form, as in the acidic environment of most cigarette smoke, nicotine crosses membranes poorly. As a consequence, there is virtually no buccal absorption of nicotine from cigarette smoke. In contrast, smokeless tobacco products are buffered to an alkaline pH that facilitates absorption.

The rate of absorption of nicotine from smokeless tobacco depends on the product and the route of administration. With fine-ground nasal snuff, blood levels of nicotine rise almost as fast as those that are observed after cigarette smoking *(4)*. The rate of nicotine absorption with the use of oral snuff (and presumably chewing tobacco) is more gradual *(5)*.

People who use oral smokeless tobacco, particularly those who chew tobacco, generate large amounts of saliva, some of which is expectorated and some of which is swallowed. Due to first pass metabolism in the liver following absorption from the intestines, the bioavailability of swallowed nicotine is approximately 30 percent *(6)*. By changing how much is chewed, how much is held inside the mouth, and how much saliva is expectorated or swallowed, the user of smokeless tobacco has considerable control over the dose of nicotine that is absorbed.

Distribution of Nicotine

Smoking is a unique form of drug administration in that entry into the circulation is through the pulmonary rather than the portal or systemic venous circulations. The lag time between smoking and the appearance of nicotine in the brain is even shorter than after intravenous injection. Nicotine enters the brain quickly, but then brain levels decline rapidly as it is distributed to other body tissues. The rapid brain uptake of nicotine from smoking allows easy puff-to-puff titration of desired nicotine effects and partly may explain the highly addictive nature of cigarette smoking.

In contrast, the concentrations of nicotine that enter the brain from smokeless tobacco use are likely to be lower *(6)*, and the pharmacologic effects may differ. The rate of exposure to psychoactive drugs is an important determinant of their effects. Thus there could be differences in the effects of nicotine that is taken by smoking compared to using smokeless tobacco, even with the same average body concentrations of nicotine.

Nicotine Elimination

Nicotine is rapidly and extensively metabolized primarily in the liver but also to a small extent in the lung and kidney. Renal excretion depends on urinary pH and urine flow and accounts for 2 to 35 percent of total elimination *(7,8)*. The half-life of nicotine averages 2 hours, although there is considerable individual variability that ranges from 1 to 4 hours *(9)*. The major metabolites of nicotine are cotinine and nicotine-N-oxide. Neither metabolite appears to be pharmacologically active *(8)*. Because of its long half-life, cotinine is commonly used as a marker of nicotine intake in survey and cessation studies. It should be recognized, however, that first pass metabolism of swallowed nicotine may result in cotinine levels that are disproportionately higher than nicotine levels with the use of smokeless tobacco compared to the use of cigarettes.

Nicotine and Cotinine Levels in Users of Smokeless Tobacco

Blood or plasma concentrations of nicotine in cigarette smokers who were sampled in the afternoon generally ranged from 10 to 50 ng/ml *(10)*. The increment in blood nicotine concentration after a single cigarette is smoked ranges from 5 to 30 ng/ml, depending on how the cigarette is smoked *(11,12)*.

In users of moist oral snuff or chewing tobacco, the levels of nicotine increase an average from 2.9 to 21.6 ng/ml during 8 hours of repeated use *(1)*. In habitual users of nasal snuff, blood levels of nicotine increased on average by 12.6 ng/ml after a single dose of snuff, and levels averaged 36 ng/ml after multiple doses *(4)*. Similarly, blood cotinine concentrations averaged 197 ng/ml and 411 ng/ml in groups of oral and nasal tobacco users, respectively, compared to an average cotinine level of 300 ng/ml for cigarette smokers described in many studies *(1,4)*. These comparisons indicate that the intake of nicotine and nicotine levels in habitual users of smokeless tobacco are similar to those that are observed in habitual cigarette smokers.

Time Course of Nicotine Turnover During Daily Tobacco Use

Tobacco use is commonly considered to be a process of intermittent dosing of nicotine, which in turn is rapidly eliminated from the body. Smoking produces considerable variations from highest to lowest blood nicotine levels from one cigarette to the next cigarette. However, consistent with a half-life of 2 hours, nicotine accumulates over 6 to 8 hours of regular smoking, and nicotine levels persist overnight, even as the smoker sleeps *(13)*. The same accumulation is probable with repeated smokeless tobacco use. Thus as with the smoker, the smokeless tobacco user may be exposed to nicotine for 24 hours each day.

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NICOTINE ADDICTION ASSOCIATED WITH SMOKELESS TOBACCO USE

Background and Definitions

Clinical observations and data, historical anecdotes, and sworn testimony all support the conclusion that some users of smokeless tobacco are unable to abstain permanently from smokeless tobacco, even when ill health is apparent *(1)*. Such observations suggest that smokeless tobacco use can become a form of drug addiction or dependence.*

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* The terms "addiction and dependence" will be used almost interchangeably throughout this section. While many argue the value of one of these terms over the other, it is important to note that in the context of this chapter they address the question of whether nicotine resulting from smoking or smokeless tobacco use leads an individual to lose voluntary control over his or her use of tobacco products (i.e., does the drug cause either dependence or addiction).

This section of the report will evaluate the scientific evidence that smokeless tobacco is an addictive substance whose use results in drug dependence. *Drug dependence* as used in this review is defined in accordance with the World Health Organization's Expert Committee on Drug Dependence *(2)* and other recognized sources *(3)*. Drug dependence is substance-seeking behavior that is controlled by the activity of a constituent drug in the central nervous system and displaces other behavior such that drug seeking assumes greater priority. Tolerance and physiologic withdrawal may or may not be present *(2,3)*, and the severity of dependence may vary considerably among individuals.

The scientific standard for classifying a drug as likely to cause addiction or dependence is based on the degree to which "abuse liability" and "physical dependence potential" are present. Both terms are accepted terminology of the Committee on Problems of Drug Dependence and the Addiction Research Center (ARC) of the National Institute on Drug Abuse *(4,5)*[*] and are commonly accepted to refer to drugs whose actions are mediated by the central nervous system. *Abuse liability* refers to drug effects that contribute to compulsive self-administration, often in the face of excessive financial cost, physical and social dysfunction, and the exclusion of more socially acceptable behaviors *(5,6)*. *Physical dependence potential* (also referred to as physiological dependence potential) pertains to the direct physiologic effects that are produced by the repeated administration of a drug that results in neuroadaptation *(3,4)*. Neuroadaptation is characterized by demonstrated tolerance to the effects of the drug and the occurrence of physiologic withdrawal signs following the termination of drug administration.

Physiologic or physical dependence, as evidenced by physiologic and behavioral rebound (withdrawal) effects, is neither necessary nor sufficient to define drug dependence *(3,5)*. Nevertheless, the process of drug dependence and abuse entails physical components, including physical interactions between drug and tissue in the central nervous system (specific receptors in the case of some drugs such as nicotine and opioids) that are critical.[†]

Three lines of evidence are important to assess the abuse liability and physical dependence potential of smokeless tobacco use. The first involves inference from the systematic comparison of tobacco use (including smokeless forms) to the use of prototypic dependence-producing drugs (e.g., alcohol, morphine, and cocaine) to determine whether the patterns of tobacco use, as well as the behavioral and physiologic effects of such use, are similar to those of the prototypic dependence-producing drugs. This issue is discussed below in the section entitled "Commonalities Between Tobacco Use and Other Dependence-Producing Substances."

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[*] The Committee on Problems of Drug Dependence is an internationally comprised body of researchers who provide advisory information to organizations, including NIDA, the World Health Organization, the Drug Enforcement Administration, and the pharmaceutical industry, regarding the understanding of drug dependence and the identification of dependence-producing drugs. The ARC is the intramural research laboratory of the National Institute on Drug Abuse, which has as a portion of its mandated responsibility the task of assessing the abuse liability and physical dependence potential of substances. For nearly 50 years, the ARC has been the largest research facility in the United States devoted to the problem of drug abuse and addiction.

[†] A concept that is central to many discussions of drug dependence is that the substance produces damage or debilitation. This aspect of tobacco dependence will not be addressed here because extensive data already exist indicating the actual toxicity of tobacco and there is widespread recognition even by tobacco users that the substance is harmful.

The second line of evidence emerges from recent studies in which nicotine was evaluated using the same methods and criteria that have been used to evaluate any substance that is suspected of causing abuse and physical dependence. This deductive approach evaluates whether nicotine meets rigorous experimental criteria as a drug that has substantive liability for abuse and physical dependence potential. This issue is discussed in the section entitled "Experimental Studies of the Abuse Liability and Dependence Potential of Nicotine."

The third line of evidence comes from recently completed studies that involve direct assessments of the abuse liability and dependence potential of orally given nicotine. Examination of these studies provides indications of whether the consumption of nicotine through oral forms of administration delivers pharmacologically active quantities of nicotine to the bloodstream and whether smokeless tobacco itself meets specific criteria for abuse liability and dependence potential. This issue is discussed in the section entitled "Evidence That Orally Delivered Nicotine (Including Smokeless Tobacco) Has a Liability for Abuse and a Potential to Produce Dependence."

Taken together, the first and second lines of evidence support the conclusion that smokeless tobacco contains an addictive substance. The third line of evidence suggests that delivery of the addictive substance (nicotine) in the form of smokeless tobacco does not alter its addictive properties.

Commonalities Between Tobacco Use and Other Addictive Substances

The assertion that tobacco use can occur as a form of drug addiction rests firmly on the observed commonalities between the use and effects of tobacco and the use and effects of addictive substances such as alcohol, opium, and coca. Systematic reviews of these commonalities have been published *(7-11)*, and the major points that tobacco and addictive substances have in common are as follows:

- A centrally (CNS) active substance (drug) is delivered.
- Discriminative (subjective) effects are centrally mediated.
- The substance (drug) is a reinforcer for animals.
- The patterns of acquisition and maintenance of substance ingestion are orderly.
- The patterns of self-administration of the substance are orderly.

- The patterns of self-administration of the substance vary as a function of the dose that is consumed.
- Tolerance to the behavioral and physiologic effects of the substance develops with repeated use (neuroadaptation).
- Therapeutic effects may be produced by the substance.
- The treatment of addiction resulting from the substance (drug) involves similar strategies.

The evidence concerning tobacco and these factors is presented in the following subsections.

Tobacco Use Delivers a Centrally Active Substance—Nicotine

The fundamental commonality between tobacco use and the use of known addictive substances is the delivery of a chemical to the central nervous system. The primary agent in tobacco, nicotine, is delivered to the central nervous system in all commonly used forms of tobacco *(12)*. The fact that cigarette smokers will substitute smokeless tobacco, when cigarettes are not available or when the use of combustibles is restricted, certainly suggests that different forms of tobacco use produce acceptably similar effects for the user *(13)*.

Discriminative Effects of Nicotine Are Centrally Mediated

Nicotine, like other drugs of abuse, produces dose-related effects in animals, which can be attenuated by centrally acting antagonists *(14-16)*. When the animals confuse these effects with other drugs (i.e., effects partially generalize to other drugs of abuse), it is more likely to be a drug like amphetamine rather than a sedative-like drug *(17)*. These findings are also consistent with data derived from studies with humans in which the dose-related effects of intravenously given nicotine were attenuated by mecamylamine pretreatment *(18)*.

Nicotine Is a Reinforcer for Animals

Most drugs that are abused by humans are voluntarily self-administered when they are made available to animals in laboratory studies; in other words, the drug serves as a reinforcer or a reward *(19,20)*. Such findings confirm that the physiologic effects of the drug in the central nervous system are sufficient for the substance to control behavior by virtue of its reinforcing effects. Definitive studies that were undertaken in the early 1980's support this statement. As seen in table 1, nicotine has now been shown to function as a reinforcer for five nonhuman animal species and under a variety of conditions *(21,22)*. Furthermore, its functional behavioral effects are similar to those engendered when other drugs of abuse (e.g., cocaine) serve as reinforcers.

Patterns of Acquisition and Maintenance of Tobacco Use Are Orderly

The use of tobacco, like that of prototypic addictive substances, is often initiated due to peer influences *(23)*. The contribution of social

TABLE 1.—Summary of Reports in Which Nicotine Was Available Under Intravenous Drug Self-Administration Procedures

Study	Species	Reinforcement Schedule	Main Finding	Comment
Deneau and Inoki (1967)	Rhesus Monkey	Fixed-ratio 1 (FR 1). Several doses of nicotine were tested.	Two monkeys initiated self-administration (S-A); the others required a priming procedure.	Currently accepted criteria to assess reinforcing efficacy were not achieved.
Yanagita, Ando, Oinuma, and Ishida (1974)	Rhesus Monkey	Experiment 1: FR 1. Several doses of nicotine and lefetamine and saline were tested.	Nicotine did not serve as a reinforcer when compared to saline or lefetamine.	—
		Experiment 2: FR 1. Several doses of nicotine were continuously available for at least 4 weeks.	Stable rates of nicotine S-A occurred in most subjects but were not clearly related to dose.	No direct test of reinforcing efficacy was done.
		Experiment 3: Progressive ratio (PR) procedures. Two doses of nicotine and saline and three doses of cocaine were tested.	At 0.2 mg/kg nicotine, response rates slightly exceeded those maintained by saline or the lowest cocaine dose (0.03 mg/kg).	Nicotine was marginally reinforcing when compared to cocaine.
Lang, Latiff, McQueen, and Singer (1977)	Hooded Rat	FR 1. Nicotine and saline were tested in food-sated and food-deprived rats.	In food-deprived (but not food-sated) rats, nicotine was a reinforcer when compared to saline.	—
Singer, Simpson, and Lang (1978)	Hooded Rat	Concurrent [(FR 1: nicotine), (Fixed-time 1 min.: food pellet)] in food-deprived rats. Subsequently, the rats were food-sated.	Food satiation decreased rate of nicotine S-A, however, nicotine was a reinforcer in both conditions.	Results were similar to those obtained when rats were similarly tested with ethanol.

TABLE 1.—Continued

Study	Species	Reinforcement Schedule	Main Finding	Comment
Griffiths, Brady, and Bradford (1979)	Baboon	FR 160 followed by 3-hr. timeout. Several doses of nicotine and saline were substituted for cocaine.	Number of nicotine injections per day did not exceed that of saline.	Caffeine, ephedrine, and a variety of other similarly tested stimulants did serve as reinforcers relative to saline in this paradigm.
Hanson, Ivester, and Moreton (1979)	Albino Rat	FR 1. Several doses of nicotine and saline were tested.	Mecamylamine (centrally acting antagonist) but not pentolinium (peripherally acting antagonist) altered S-A behavior.	Group data suggest that nicotine was a reinforcer; however, there was no clear dose-effect curve.
Latiff, Smith, and Lang (1980)	Hooded Rat	Conc (FR 1: injection) (FT 1 min.: food pellet). Several doses of nicotine and saline were tested.	Nicotine was a reinforcer relative to saline. Urine pH manipulations had mild effects on rate of S-A only during initial exposure to nicotine.	Rate of S-A was inversely related to dose during initial exposure to nicotine but not after nicotine S-A was established.
Smith and Lang (1980)	Hooded Rat	FR 1. One dose of nicotine and saline were tested.	Nicotine was established as a reinforcer both with and without a concurrent food delivery schedule in food-deprived but not food-sated rats.	—
Goldberg, Spealman, and Goldberg (1981)	Squirrel Monkey	Second order schedule FI 1 or 2 min. (FR 10: stimulus) followed by 3-min. timeout. One dose of nicotine and saline was tested.	Nicotine maintained high rates of responding. Rates decreased markedly when (1) saline replaced nicotine, (2) the brief stimuli were omitted, and (3) subjects were pretreated with mecamylamine.	Demonstrated the importance of ancillary environmental stimuli in maintaining high rates of responding.

TABLE 1.—Continued

Study	Species	Reinforcement Schedule	Main Finding	Comment
Ator and Griffiths (1981)	Baboon	FR 2 followed by 15-sec. timeout. Several doses of nicotine and saline and cocaine were tested.	Nicotine was marginally reinforcing compared to saline across a narrow dose range.	Initial dose-response curve was inverted U-shaped, and final dose-response curve was flat (from abstract of study).
Dougherty, Miller, Todd, and Kostenbauder (1981)	Rhesus Monkey	FI 16 and second order FI 1 min. (FR 4: stimulus). Several doses of nicotine and saline were tested.	Nicotine maintained higher rates of S-A than saline under the FI and second order schedules but was only a marginally effective reinforcer when continuously available.	Establishment of nicotine as a reinforcer required several months using procedures that typically require only a few days to establish cocaine or codeine as reinforcers.
Goldberg and Spealman (1982)	Squirrel Monkey	FI 5 min. Several doses of nicotine and cocaine and saline were tested.	Nicotine and cocaine were qualitatively similar reinforcers when compared to saline. Cocaine maintained higher rates of responding in one of two monkeys. Mecamylamine pretreatment reduced rates of nicotine S-A.	This study also showed that nicotine could serve as a punisher similar to electric shock.
Singer, Wallace, and Hall (1982)	Long-Evans Rat	CONC [FR 1: nicotine) (FT 1 min.: food pellet)]. One dose of nicotine was tested.	A group of rats with 6-OHDA lesions in the nucleus accumbens S-A nicotine at lower rates than a sham-lesioned group.	Extended the range of scheduled-induced behaviors that are inhibited by such lesions.

TABLE 1.—Continued

Study	Species	Reinforcement Schedule	Main Finding	Comment
Spealman and Goldberg (1982)	Squirrel Monkey	Second order FI 1, 2, or 5 min. (FR 10 stimulus) and FI 5 min. schedules were tested. Several doses of nicotine and cocaine and saline were tested.	Nicotine and cocaine maintained similar patterns of responding on the schedules. Nicotine, but not cocaine S-A, decreased to saline-like rates when animals were pretreated with mecamylamine.	Nicotine's reinforcing efficacy was comparable to that of cocaine.
Risner and Goldberg (1983)	Beagle Dog	FR 15 followed by 4 min. timeout. Several doses of nicotine, cocaine, and saline were tested. Progressive ratio schedule was used.	Nicotine and cocaine maintained qualitatively similar patterns of responding and were reinforcers relative to saline. Mecamylamine pretreatment reduced nicotine but not cocaine S-A.	Cocaine maintained substantially greater response rates than nicotine.
Henningfield, Miyasato, and Jasinski (1983)	Human	FR 10 followed by 1 min. timeout. Several doses of nicotine and saline were tested.	Number of nicotine injections generally exceeded number of saline injections and were inversely related to nicotine dose. Post-session cigarette smoking was suppressed by nicotine.	Nicotine produced subjective effects similar to those produced by intravenous cocaine and had both reinforcing and punishing effects.
Goldberg and Henningfield (1983)	Human and Squirrel Monkey	FR 10 followed by 1 min. timeout. Several doses of nicotine and saline were tested.	Patterns of responding were qualitatively similar in both species. Number of nicotine injections exceeded number of saline injections in 3 of 4 human and 3 of 4 monkey subjects.	In both the human and monkey subjects, there was evidence that nicotine functioned with both reinforcing and punishing properties.

support to the initiation of tobacco use may be even greater than with illicit drugs, because family members, other social models, and advertising often tolerate, approve, or promote tobacco use while disapproving the use of some nonprescription drugs *(24)*. Also, as is the case with addictive drugs, an accelerated pattern of development of tobacco use has been observed, which is followed by relatively stable drug intake. Initially, the level of consumption increases gradually from the first day of use until some point, perhaps several years later, when it becomes relatively stable over time. Although many factors can operate to produce such a biphasic pattern of intake, it is generally assumed that tolerance and learning factors account for the gradual acceleration and that a level of optimum drug effect combined with toxicity and adverse effects at higher doses takes over to produce the stabilization phenomenon. A preliminary survey, conducted at Johns Hopkins University, indicates that nicotine, whether administered as cigarette smoke or smokeless tobacco, does not differ from other drugs in this regard. That is, tobacco users tend to begin smoking a few cigarettes a day or consume a portion of a container of smokeless tobacco each day and gradually increase consumption levels over a period of months or even years before they stabilize the amount they finally use (personal communication, J.E. Henningfield).

Patterns of Tobacco Self-Administration Are Orderly

Daily patterns of cigarette smoking are orderly. Addicted smokers tend to smoke their first cigarette within 30 minutes of waking from a night of sleep and find it difficult to abstain from tobacco use for more than a few hours *(25)*. If smoking behavior is relatively unconstrained, regular patterns develop that closely resemble those of psychomotor stimulant self-administration in animals *(20)*. Similar orderly patterns of tobacco self-administration are evident with cigarette smoking by humans. Several studies have demonstrated that across successive puffs on a cigarette, puff duration decreases and interpuff intervals tend to increase *(26,27,28,29)*, although these changes are multifactorially determined *(30)*. Anecdotal reports by smokeless tobacco users suggest that while consumption patterns are necessarily different (e.g., some keep a plug in their mouth almost continually during their waking hours) they are no less regular and orderly.

Tobacco Self-Administration Varies as a Function of Nicotine Dose

The effective dose of a substance may be varied by changing the quantity of drug per unit (the unit dose), by pretreating the individual (animal or human) with either an agonist or antagonist, or by altering the rate of elimination of the substance. Studies that involve these three manipulations have been done extensively with other drugs and more recently with nicotine. The results across study, drug, and species are remarkably similar. For general reviews of human and animal studies see Griffiths, Bigelow, and Henningfield *(20)* and Henningfield, Lukas, and Bigelow *(31)*. See Gritz *(32)* and Henningfield *(33)* for recent reviews of the nicotine-specific literature. Over a wide range of dose levels, frequency of self-administration is inversely related to dose but drug intake is directly related to dose, reflecting partial compensatory changes *(26,32)*. Pretreatment with other agonists (or forms of nicotine) reduces drug taking, e.g., decreases cigarette smoking, *(34)* and reduces preferred nicotine concentration of tobacco smoke *(35)*. Pretreatment with antagonists initially increases drug self-administration. For example, the centrally and peripherally acting ganglionic blocker, mecamylamine, but not the peripherally acting blocker, pentolinium, increases subsequent smoking rates and increases preferred nicotine concentrations of tobacco smoke *(36,37)*. In addition, altering the elimination rate of nicotine alters the amount of nicotine that is self-administered in the form of tobacco smoke *(38)*.

There has been debate over the degree to which smokers regulate their nicotine intake, i.e., the "titration" hypothesis. It is now generally agreed that smokers do not precisely titrate their nicotine intake any more than animals titrate their intake of reinforcing drugs (except under extremely limited conditions) or humans titrate their intake of other reinforcing drugs *(20)*. However, when dose manipulations are observed and objective, sensitive dependent variables are measured in both animals and humans *(26,32,33)*, most of the studies demonstrate an increase in smoking as cigarette nicotine content falls below accustomed levels and a decrease in smoking when cigarette nicotine content is unusually high *(32)*. Kozlowski and his coworkers describe these findings in terms of a "boundry" model of dose compensation *(39)*.

Tolerance of Nicotine Develops With Repeated Use (Neuroadaptation)

The administration of most drugs of abuse results in neuroadaptation as measured by tolerance to the repeated administration of the drug and a subsequent rebound (withdrawal) when drug administration is terminated *(3)*. Tolerance to drug effects is determined either by the diminished response to repeated doses of a drug or the requirement of increasing doses to achieve the same drug effect. Tolerance to the behavioral and physiologic effects of nicotine has been studied for decades *(33)*. As is the case with other drugs of abuse, a variety of mechanisms accounts for tolerance to many of nicotine's effects, including metabolic *(40)*, behavioral *(41-43)*, and physiologic tolerance *(44-46)*. More recently, studies have shown that the effects of nicotine that are suspected to be critical to the addiction process also show tolerance with repeated dosing *(47,48)*.

Physiologic dependence on drugs is determined by showing that termination of drug administration produces a syndrome of effects that is generally opposite to those produced by drug administration. This syndrome is reversible, at least in its early stages, by administration of the

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drug. Prolonged drug abstinence (detoxification) results in ultimate return to baseline (normal) values of behavioral and physiologic functions. It is now clear that repeated tobacco administration produces physiologic dependence that is specifically due to nicotine administration. Recent data that confirm this fact are reviewed in the section on Dependence Potential of Nicotine.

Nicotine Produces Therapeutic Effects

Most drugs of abuse have specific therapeutic applications; nicotine is no exception *(48-50)*. The degree to which the therapeutic effects of nicotine depend upon the individual's history of nicotine use, as opposed to the possibility that nicotine is efficacious for preexisting conditions, remains to be investigated. Similar issues are true for other drugs of abuse as well. Pomerleau and his coworkers *(51)* have studied a variety of mechanisms by which the possibly weak, initial reinforcing effects of nicotine can be greatly strengthened by subtle effects on mood, cognition, and normal physiologic and behavioral functioning. For instance, as will be described below, nicotine may produce a small, but important, enhancement of work performance. These effects appear to be mediated by the effects of nicotine on hormonal release and regulation. The following is a brief summary of some of the effects of nicotine, considered therapeutic by tobacco users, that have been investigated.

Several studies have shown that nicotine enhances performance on a variety of cognitive tasks that involve speed, reaction time, vigilance, and concentration *(52-55)*. These effects are strongest in cigarette smokers who are deprived of cigarettes. However, such performance enhancement was also evident after the administration of nicotine to nonsmokers and was produced by increasing the nicotine dose in persons who were already smoking. Nicotine may also be a useful mood regulator by virtue of its release of norepinephrine from the adrenal medulla *(56)*. Norepinephrine release is also stimulated by excitement, exercise, sex, antidepressant drugs, and other drugs of abuse, suggesting that cigarette smoking may function pharmacologically to alleviate boredom and stress. Finally, as an anoretic *(57-60)*, nicotine appears to function in three ways: by decreasing the efficiency with which food is metabolized *(61,62)*; by reducing the appetite for foods that contain simple carbohydrates (sweets) *(63)*; and by reducing the eating that may occur in times of stress *(64)*. Nicotine may also function as an anxiolytic by reducing responsiveness to stressful stimuli and enhancing mood *(56)*. In addition, nicotine reduces aggressive responses in experimental situations *(65)*.

A well-documented therapeutic role for nicotine as a drug is evident in the treatment of tobacco abstinence for many individuals following dependent patterns of tobacco use, e.g., as assessed by the Fagerstrom Tolerance Questionnaire *(25)*. This test provides both scientific and practical evidence of the role of nicotine in tobacco dependence. It is well established that abstinence from tobacco in heavy cigarette smokers produces signs and symptoms of rebound that can be reversed by resumed tobacco use and at least partially reversed by other forms of nicotine administration *(66)*. For example, nicotine gum treatment for cigarette smoking is efficacious, although a variety of factors limit success rates *(34)*.* This drug substitution strategy is analogous to those obtained when intravenous opioid users are treated with other opioids given via other routes. For example, methadone administration may reverse signs and symptoms of opioid withdrawal, while leaving the patient feeling partially treated yet likely to relapse if not provided with an adjunctive behavioral treatment *(67)*.

Although the euphoriant properties of drugs can stand apart from collateral therapeutic actions (as is the case with morphine, amphetamine, and alcohol), attention to such drug effects may enhance the efficacy of treatment. Because nicotine, in the form of tobacco, is widely available, is relatively inexpensive, and is in a convenient form for precise dose regulation, it provides an ideal means of self-medication. These effects may contribute to the abuse liability of tobacco and are of demonstrable significance in the treatment of tobacco addiction *(51)*.

Similar Strategies Are Involved in the Treatment of Tobacco Addiction and Other Forms of Drug Addiction

If tobacco use is a form of drug addiction, then strategies of treatment of other forms of drug addiction should be applicable. Most available information and existing strategies for treatments of tobacco use are based on nonpharmacologic approaches. Such approaches have been no more useful in the treatment of tobacco dependence than in the treatment of dependence of opioids, stimulants, sedatives, or alcohol. On the contrary, experience in the treatment of drug addiction disorders makes clear the importance of addressing the pharmacologic components of the addiction *(67)*. This conclusion is strengthened by the observation that persons being treated for opioid addiction regard tobacco to be as necessary as methadone *(68)* and that persons successfully treated for other kinds of drug addiction are unable to give up tobacco *(69)*. This provides the support for the fundamental premise that tobacco addiction generally constitutes an independent health-impairing disorder. Specific treatment implications relating to cigarette smoking as a form of drug abuse are considered below.

To the extent that tobacco use is similar to other forms of drug abuse, treatment strategies that are used for drug abusers may be applied to the treatment of cigarette smoking. Although it is not the purpose of this chapter to describe in detail the treatment for cigarette smoking, a few commonalities, as well as differences, are worth mentioning. Four basic pharmacologic treatments for drug abuse provide the advantage of licit administration of an agent controlled by a certified clinician. These involve substitution therapy (e.g., methadone for opiate dependence) in which a more manageable form of the drug is provided according to a prearranged maintenance protocol; blockade therapy (e.g., naltrexone for opiate dependence) in which the effects of the abused drug are blocked by pretreatment with an antagonist; and nonspecific supportive therapy in which the patient is treated symptomatically, exemplified by the temporary use of benzodiazepines during alcohol detoxification *(67)*. All three approaches have been used in the treatment of cigarette smoking with varying degrees of success *(48)*. A fourth strategy of pretreating the patient with a drug that results in adverse side effects when the subsequent abused drug is taken (e.g., treatment of alcoholism with disulfiram) has not been systematically explored with tobacco.

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* These therapeutic effects are produced by nicotine chewing gum, an orally administered form of nicotine that is approved by the Food and Drug Administration (FDA). The gum is obtainable in the United States by prescription only and is commonly used by physicians to help individuals quit smoking.

The most recent, widely used treatment for cigarette smoking, and the first of those recognized as efficacious by the FDA, is modeled directly after the treatment of heroin addiction by methadone substitution. This treatment is nicotine gum substitution *(70)*. It is a practical application of the postulate that tobacco use is basically a form of drug addiction on nicotine. This recognition is especially relevant here, because smokeless tobacco is an oral form of nicotine. All of the relevant therapeutic data support the premise that compulsive tobacco use entails nicotine addiction, which in the form of tobacco exposes the user to health hazards, and that therapeutic strategies paralleling those for other forms of drug abuse are effective in treatment. Differences appear to be principally related to the social tolerance of tobacco addiction, relative to other forms of drug addiction, which contribute to greater difficulty in treating this form of drug abuse.

Summary of Commonalities Between Tobacco and Prototypic Addictive Drugs

The preceding review has shown that tobacco shares many points in common with prototypic addictive drugs. These similarities provide a strong conceptual basis for the categorization of tobacco as an addictive drug. The behavioral process is orderly, tobacco self-administration results in the delivery of a centrally active drug (nicotine), and the drug appears to be the major determinant in the control of the compulsive behavior of tobacco self-administration. These findings are consistent with those expected with animal and human subjects, as determined across a broad range of studies of drugs of abuse *(20)*.

In summary, tobacco, opium, and coca produce different effects but share a number of important similarities. Whereas large doses of opioids can produce a debilitating sedation, high doses of coca alkaloids (cocaine HCl) produce levels of behavioral excitation that are not normally produced by tobacco; but the intake of all of these substances leads to compulsive use. Compulsive use and the other commonalities described in the preceding subsections provide compelling evidence that tobacco use can be a form of drug dependence or addiction. The next major question is what element(s) of tobacco are critical to controlling the behavior of the user. The conceptual leap from habitual behavior to drug abuse and addiction can be made only on the basis of evidence that a specific psychoactive drug is critical to the behavior. The next section on the abuse liability and dependence potential of nicotine will address this question.

Experimental Studies of the Abuse Liability and Physical Dependence Potential of Nicotine

The comparison of tobacco to prototypic addictive drugs is the basis for concluding that compulsive tobacco use is a form of drug dependence behavior in which nicotine plays an important role. To test this hypothesis further, it should be possible to show that nicotine is an abusable substance even in the absence of the many stimuli associated with cigarette smoking. This can be done by evaluating nicotine in accordance with methods and criteria that have been used to assess any substance that is suspected of causing abuse and physical dependence. One-half century of research at the NIDA Addiction Research Center, and research in other laboratories, has produced valid and reliable experimental methods to evaluate a substance's potential to cause abuse and to produce physical dependence. The methods are empirically based on generally accepted examples of drug addiction, most notably opioid dependence (e.g., morphine) and, to a lesser degree, psychomotor stimulant dependence (e.g., cocaine) and sedative dependence (e.g., barbiturates and alcohol). These methods encompass standards for assessing the two dimensions of drug addiction—abuse liability and physical dependence potential. The evidence that is related to the abuse liability and physical dependence potential of nicotine is presented below.

Abuse Liability of Nicotine

Abuse liability refers to drug effects that contribute to compulsive self-administration, often in the face of excessive financial cost, physical and social dysfunction, and the exclusion of more socially acceptable behaviors *(5,6)*. In other words, it entails those effects of a substance that contribute to diminution of voluntary control over the use of the substance by the individual.

Objective methods to assess abuse liability are available and have been used to assess diverse agents *(5)*. These methods have been readily adapted to studies of nicotine abuse liability, with consideration given to the fact that nicotine has more rapid effects than many other drugs of abuse.

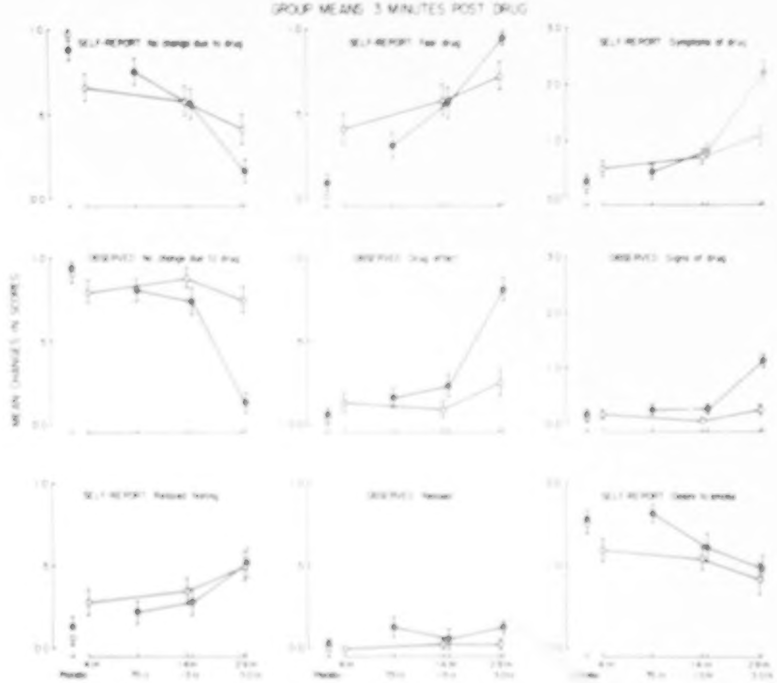
The hypothesis is that nicotine is psychoactive and serves as a euphoriant and reinforcer. Psychoactivity and euphoria are determined by assessing the pharmacodynamic subjective effects of single doses of the drug ("single-dose" or "abuse liability" studies) and are validated by observed behavioral and physiologic responses. Reinforcing efficacy is determined by assessing the ability of the drug to strengthen and maintain orderly patterns of behavior when the subject is permitted access to the drug (i.e., the prototypic "self-administration" study).

Pharmacodynamic Effects of Nicotine. In human studies of nicotine-related psychoactivity, volunteers are given a range of doses of the test compound and placebo under double-blind conditions. Persons with histories of drug abuse are used because they can accurately discriminate compounds with a potential for abuse and can compare the effects of the compounds to those of abuse drugs *(5)*. In one study, three doses of nicotine were given both intravenously and in the form of tobacco smoke under controlled conditions *(71)*. Nicotine produced a similar profile of effects (figure 1). Self-reported (subjective), observer-reported (behavioral), and physiologic variables were measured before, during, and after drug administration. In brief, nicotine was shown to be psychoactive, as evidenced by the reliable discrimination of nicotine from placebo. Self-reported effects of nicotine peaked within 1 minute after administration (by either route) and dissipated within a few minutes: peak and duration of response were directly related to the dose.

The two hallmark indicators of euphoria in such studies are the Liking Scale (Single Dose Questionnaire) and the Morphine Benzedrine Group (MBG) Scale (Addiction Research Center Inventory [ARCI]) (5). Responses on the 5-point Liking Scale, which asked how much the drug was liked (0 = "not at all," 4 = "an awful lot") are presented in figure 2. Nicotine produced responses on the Liking Scale similar to those of morphine and *d*-amphetamine. MBG Scale scores of the ARCI were consistent with the Liking Scale data, confirming that nicotine, given by both routes of administration, was a euphoriant. In another comparison between drugs, subjects more frequently identified nicotine injections as cocaine.

Similar results for intravenous and inhaled nicotine were also obtained on several physiologic measures, including pupil diameter, blood pressure, and skin temperature. These data confirmed that nicotine, given in either tobacco smoke or intravenously, was the critical pharmacologic compound accounting for these effects of tobacco smoke. A subsequent study showed that nicotine's subjective and physiologic effects could be partially blocked by pretreating the subjects with the antagonist mecamylamine *(18)*. Results of studies with animals also indicate that nicotine produces discriminable effects, and the data suggest that animals identify nicotine as being more similar to cocaine than to placebo or pentobarbital, but not identical to cocaine *(17)*.

FIGURE 1.—This figure is a summary of the data from a study of the liability of nicotine delivered as tobacco smoke (filled symbols-IN) or intravenous injections (open symbols-IV). Dose is presented on the horizontal axes. Even with a controlled smoking procedure, nicotine dose administration via cigarette smoke is more variable (producing flatter dose-response functions) than when given intravenously. Also, important effects of nicotine are covert though reliable and orderly (e.g., relaxed feelings, symptom scores). The finding that a low dose of tobacco smoke was more effective in reducing desire to smoke than a low dose of intravenous nicotine is consistent with the fact that satisfaction from smoking is also due to stimuli provided by the cigarette and the smoke.



Self-Administration of Nicotine. The second abuse liability dimension uses the "self-administration" procedure to examine the conditions under which a subject will voluntarily take the drug. Self-administration studies determine whether the drug serves as a biologically effective, positive reinforcer (or reward). Variants of these strategies are conducted in both animal and human subjects, thereby providing a means of establishing the biologic generality of the phenomena, while controlling the possible confounding influence of personality, social, or cultural variables. A high degree of concordance between findings from animal

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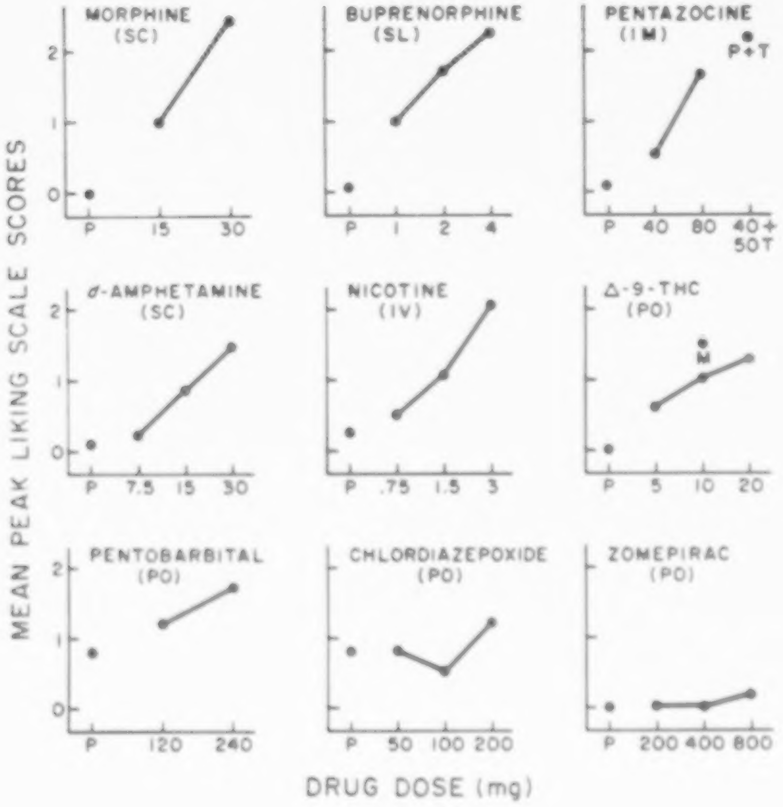
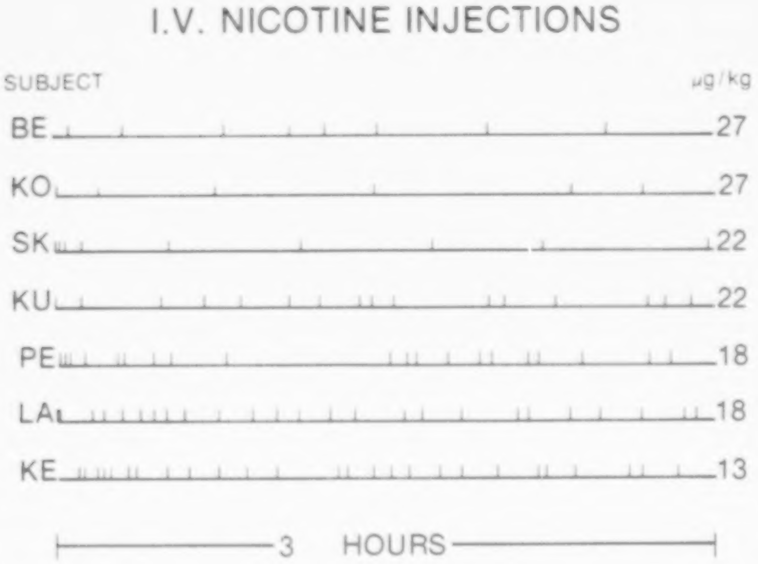
FIGURE 2.—This figure presents data from a series of abuse liability studies conducted at the Addiction Research Center. The findings that Liking Scale scores are directly related to dose and exceed placebo values are important in identifying dependence-producing drugs. Intravenous nicotine produced the same elevated dose-response function as highly addictive narcotics (e.g., morphine) and a prototypic stimulant (*d*-amphetamine). These data are also consistent with the lower abuse liability of chlordiazepoxide and almost negligible abuse liability of zomepirac. Administration of intravenous cocaine results in a function similar to that shown for intravenous nicotine, except that the cocaine dose levels must be increased by a factor of 5 to 10.



and human studies has been established over a wide range of drugs *(20)*. Therefore, this section focuses on the results of studies using human volunteers.

The methods developed in animal studies can be used to assess whether the pharmacologic activity of a drug maintains self-administra-

FIGURE 3.—This figure shows the patterns of nicotine self-administration that occurred when volunteer cigarette smokers were given the opportunity to take injections of nicotine, but not smoke cigarettes, during 3-hour tests. The amount of nicotine available was roughly comparable to that obtained by smoking cigarettes. The subjects smoked less following sessions in which they took nicotine than following sessions in which only saline (the placebo) was available.



tion paralleling drug seeking and drug taking by individuals in the natural environment or "real world." The strategy is particularly useful in studies of nicotine, because it precludes confounding by other stimuli that are associated with tobacco smoke inhalation (e.g., the tobacco brand, smell of the smoke, and lighting-up rituals).

In one such study, tobacco-deprived volunteers were tested during 3-hour sessions in which 90 presses on a lever resulted in either a nicotine or placebo injection *(72)*. All six subjects voluntarily self-administered nicotine (figure 3). Patterns of self-administration (injections) were similar to those observed when human subjects smoke cigarettes and when rhesus monkeys take intravenous amphetamine injections in comparable experimental situations *(20)*.

One subject, who lacked a history of drug abuse, exhibited an acquisition pattern of nicotine self-administration that developed gradually over several sessions. The pattern was a prototypic example of drug abuse development. Double-blind substitution of saline for nicotine resulted in cessation of the self-injection behavior of subject KO (figure 3). Subjects who were given access to both nicotine and placebo concurrently (by pressing alternate levers) chose nicotine, confirming that nicotine had come to serve as a positive reinforcer *(73)*. These data indicate that the pharmacologic activity of nicotine was critical to the maintenance of the behavior.

Nicotine self-administration has been studied in a variety of nonhuman species under a variety of experimental conditions *(74)*. As noted earlier, recent results confirm that nicotine can function as an effective reinforcer although the conditions under which it serves as a reinforcer for animals are more restricted than those for morphine or cocaine *(21)*. Nicotine self-administration via cigarette smoke or smokeless tobacco may provide ideal confluences of conditions for the establishment and maintenance of nicotine dependence in humans *(33)* with the presence of immediate and abundant peripheral taste and olfactory stimuli *(75)*.

Implications of Pharmacodynamic and Self-Administration Studies. The results of the pharmacodynamic and self-administration studies provide direct evidence that nicotine itself, and apart from its being presented in combination with all of the orosensory properties of tobacco smoke, is an abusable drug. That is, nicotine meets the criteria of being psychoactive: it serves as a euphoriant and as a reinforcer. These findings strongly suggest that nicotine parallels other drugs (e.g., morphine in opium use, cocaine in coca leaf use, and ethanol in alcoholic beverage consumption) in its ability to maintain self-administration. The findings are of sufficient strength that the relevant public health implications have already been incorporated into issues of public health policy by the former Director of the National Institute on Drug Abuse, Dr. W. Pollin *(76)*, the U.S. Public Health Service *(77)*, and the former Secretary of the Department of Health and Human Services, Mrs. M. Heckler *(78)*.

Physical Dependence Potential of Nicotine

Physical dependence potential (also referred to as physiological dependence potential) pertains to the direct physiologic effects that are produced by the repeated administration of a drug that results in neuroadaptation *(3,4)*. Neuroadaptation is characterized by demonstrated tolerance to the effects of the drug and the occurrence of physiologic withdrawal signs following the termination of drug administration.

Physical dependence potential studies are conducted according to standardized tests, using methods such as the substitution approach in which an active drug is removed and replaced with either a placebo or another form of the drug *(5)*. Although many studies on the effects of tobacco abstinence on mood, behavior, and physiologic functions have been conducted, until recently, the classic "direct addiction" or "substitution" methodologies had not been used to study the physical dependence potential of nicotine *(79)*.

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The absence of such studies and the fact that many critical markers of tobacco abstinence are not overt or easily measured (e.g., change in affect, EEG, and cognitive performance impairment) have led to questions about the severity of the tobacco withdrawal syndrome *(33)*. However, as shown below, abstinence from chronic tobacco or oral nicotine use is followed by a syndrome of behavioral and physiologic changes that are orderly, replicable, specific to nicotine, and of functional consequence in relapse to tobacco following abstinence. The apparent absence of withdrawal symptoms among some people is not inconsistent with the finding that nicotine has the *potential* to produce physical dependence. As is true for users of opiates (e.g., heroin), the magnitude of the withdrawal syndrome is related to a variety of factors such as dosage and individual predispositions *(80)*.

Definition of Tobacco Withdrawal. There are abundant data indicating neuroadaptation to tobacco use, showing that this adaptation is at least partially nicotine specific and that termination of chronic tobacco use produces a behavioral and physiologic rebound or withdrawal syndrome *(33)*. This has been stated in the Diagnostic and Statistical Manual (DSM) of the American Psychiatric Association (APA) as follows *(81)*:

> Tobacco Withdrawal (APA, DSM, III, 1980). The essential feature is a characteristic withdrawal syndrome due to recent cessation of or reduction in tobacco use that has been at least moderate in duration and amount. The syndrome includes craving for tobacco, irritability, anxiety, difficulty concentrating, restlessness, headache, drowsiness, and gastrointestinal disturbances. It is assumed that this syndrome is caused by nicotine withdrawal, since nicotine is the major pharmacologically active ingredient in tobacco.
>
> Withdrawal does not occur with all smokers; but in many heavy cigarette smokers, changes in mood and performance that are probably related to withdrawal can be detected within two hours after the last cigarette. The sense of craving appears to reach a peak within the first 24 hours after the last cigarette, thereafter gradually declining over a few days to several weeks. In any given case it is difficult to distinguish between a withdrawal effect and the emergence of pychological traits that were suppressed, controlled, or altered by the effects of nicotine.

This definition by the American Psychiatric Association represents a reasonable consensus from various reviews of the literature on cigarette smoking and physiologic dependence on tobacco *(3,13,32,82,83)*. It is based on experimental data and clinical observations from cigarette smoking treatment studies demonstrating that certain signs and symptoms are of unusually high prevalence during the first few days of abstinence. Decreased heart rate and blood pressure have been studied experimentally *(84)*, as well as changes in cortical EEG potentials *(85,86)*, changes in urine catecholamine excretion *(87)*, and weight gain *(57)*. Other possible concomitants of tobacco withdrawal reported clinically include headaches, gastrointestinal disturbances, insomnia, and fatigue *(82,87)*. A variety of behavioral effects occurs when tobacco or nicotine administration is abruptly terminated in human and animal subjects, including increased irritability, aggressiveness, and anxiety; performance also is impaired in various psychomotor and learning tests such as simulated driving, vigilance, and paired-associate learning *(88-90)*. Self-reported desire to smoke cigarettes ("craving") increases sharply for about 1 day following abstinence, then gradually declines over the course of about 1 week to a lesser level *(91)*. Most of these signs and symptoms of withdrawal subside over 1 to 2 weeks; however, some former tobacco users report that the desire to smoke may recur for many years and may be evoked by specific environmental stimuli that were previously associated with smoking, such as after meals or in selected social situations. This, too, parallels the powerful conditioning phenomena that are reported to be associated with other drugs of abuse *(92)*.

Evidence of Tobacco Withdrawal Symptoms. There is compelling evidence that acute tobacco abstinence produces a rebound (withdrawal) syndrome. This evidence comes from studies of two laboratories in which increases in low-frequency EEG bands and decreases in cortical activity were observed during the first day of tobacco abstinence *(85,86)*. These effects were immediately reversed when the subjects were allowed to smoke two cigarettes.

In a study of self-reported withdrawal symptomatology, 40 participants completed four 25-item questionnaire forms daily for 2 weeks *(93)*. Subjects were divided into two groups: totally abstinent and partially abstinent whose smoking levels were maintained at an average of 60 percent. Four symptom clusters emerged: (1) drowsiness in both groups declined over the first week and then increased over the second week, forming a U-shaped function; (2) physical symptoms (e.g., headaches and gastrointestinal disturbances) in both groups declined rapidly the first week and then remained stable across the second week; (3) psychological symptoms (e.g., anxiety and irritability) in both groups paralleled physical symptoms; and (4) craving symptoms in the totally abstinent group closely paralleled physical and psychological symptoms, whereas craving levels of the partially abstinent subjects remained elevated across the 2 weeks. The finding that partial abstinence is accompanied by persistent craving symptomatology is similar to the results of studies on the treatment of illicit opioid dependence with methadone. In these studies, low-dose methadone maintenance is associated with a persistent opioid craving *(94)*.

An important series of studies on the dependence potential of nicotine has recently been completed at the University of Minnesota *(95,96,97)*. The goals of these studies were to determine reliable and valid indicators of tobacco withdrawal by examining physical, subjective, and behavioral reactions to tobacco deprivation. The first three studies of this series evaluated the dependence potential of tobacco and established a reliable battery of measures. In a residential study, 27 smokers resided for 7 days on a research ward *(95)*. Following baseline, they were assigned to abstain from smoking or to continue smoking for 4 days. Physiologic, subjective, and behavioral measures were obtained and analyzed. The second study was conducted on a nonresidential basis to assess tobacco withdrawal in the nonlaboratory environment *(96)*. In this study, signs and symptoms of tobacco withdrawal were measured in 100 smokers. Following baseline measurements, subjects were randomly assigned to either nicotine or placebo gum, to be chewed at each subject's own rate. The subjects returned on three different occasions for assessment. The third study assessed the reliability of the tobacco withdrawal syndrome within subjects *(97)*. This study employed a modified, within-subject experimental design; baseline smoking, tobacco deprivation, return to baseline smoking, and tobacco deprivation were assessed in each subject.

The results of all three studies demonstrated that the syndrome of withdrawal that occurs reliably and consistently in chronic smokers after tobacco deprivation includes decreased heart rate, increased caloric intake/eating, an increased number of awakenings during sleep, an increased desire to smoke cigarettes, and increased confusion. Other changes that were found, but not consistently, included increased irritability and decreased vigor. A prospective examination of data from both residential and nonresidential studies revealed that there were no statistically significant differences between men and women in either number or severity of tobacco withdrawal symptoms *(98)*.

A subsequent study was designed to assess the relationship between tobacco withdrawal symptoms and pre- and post-cigarette blood nicotine levels, pre-cigarette cotinine levels, change in nicotine level from pre- to post-cigarette, half-life of nicotine, and total smoke exposure *(99)*. Twenty subjects were required to smoke cigarettes for 3 days using a portable recorder that allowed measurements of smoking topography in a nonlaboratory environment. Blood samples were drawn to determine blood nicotine and cotinine levels. Subjects abstained from cigarettes for the next 4 days. A battery of tests to measure tobacco withdrawal symptoms was administered. In general, results showed an inconsistent relationship between measures of nicotine intake and tobacco withdrawal. The most consistent finding was the relationship of the desire to smoke cigarettes to blood nicotine and cotinine levels and change in nicotine from pre- and post-cigarette; that is, the higher the nicotine and cotinine level and "nicotine boost," the greater the desire for cigarettes during abstinence.

The three initial studies that were conducted at the University of Minnesota *(95,96,97)* systematically examined the physiologic dependence produced by chronic tobacco use. This work represents a major advance in furthering the understanding of tobacco dependence. The NIDA Addiction Research Center is also nearing the completion of a series of studies on the physical dependence potential of tobacco and the degree to which oral nicotine treats the abstinence syndrome. Preliminary data analysis confirms the findings from the Minnesota studies.

Implications of Physical Dependence Potential Studies. These recent studies confirm and extend the findings of earlier investigations that demonstrated that nicotine had the potential to produce physiologic dependence. It is now known that the syndrome is orderly and is due to the administration and withdrawal of nicotine. The overt signs are more subtle than those marking opioid and sedative withdrawal, but these signs are not necessarily less important to the individual. For instance, withdrawal effects such as mood changes, performance deficits, and weight gain may be of considerable importance to the normal functioning of the individual. It is anticipated that just as detoxification and treatment of opioid and sedative dependence have benefited from improved understanding of these syndromes of withdrawal, so also may detoxification and treatment of tobacco withdrawal benefit.

Evidence That Orally Delivered Nicotine (Including Via Smokeless Tobacco) Has a Liability for Abuse and a Potential to Produce Physical Dependence

As previously indicated, moist snuff contains as much as 15.1 mg nicotine per gram; plug tobacco contains 17.2 mg per gram *(100,101)*. Lower-nicotine-containing brands exist. However, marketing efforts encourage (and users demonstrate) graduation to the higher-nicotine-containing products *(1)*. These levels of nicotine are substantial, since the relative potency of nicotine is 5 to 10 times greater than that of cocaine in producing discriminable subjective effects (1 to 2 mg of nicotine given intravenously, orally, or inhaled produces reliable behavioral and physiologic effects).

Two studies have confirmed that typical patterns of smokeless tobacco use result in the delivery of quantities of nicotine that produce plasma nicotine elevations comparable to those produced when cigarettes are smoked *(102,103)*. These studies also found that smokeless tobacco use reflected several of the indices of abuse liability and physical dependence potential. Smokeless tobacco users self-administered substantial quantities of nicotine; the patterns of smokeless tobacco use were orderly and stable; and subjective and behavioral effects may be produced from such use. More recently, a new form of smokeless tobacco, moist brown tobacco in tea bag-like pouches, was also shown to deliver pharmacologically active quantities of nicotine to the central nervous system *(104)*.

Reinforcing Properties of Nicotine in the Form of Chewing Gum

There is growing evidence that nicotine is reinforcing and has the potential to produce dependence even when absorbed through the buccal mucosa (and therefore more slowly) via chewing gum (nicotine polacrilex). One recently completed study involved the self-administration of either a nicotine- or placebo-containing chewing gum by smokers who had quit smoking *(105)*. When given a choice between placebo and nicotine chewing gum, subjects preferred nicotine to placebo and self-administered the nicotine gum throughout each day.* These data are particularly compelling, because nicotine, in the form of the nicotine polacrilex, is in an ion-bound complex. In this preparation, the nicotine is released and absorbed slowly compared to the nicotine in smokeless tobacco; and the polacrilex form of nicotine administration appears to be of relatively low abuse liability. This study also demonstrated that instructions by a physician can alter patterns of gum use and preference *(105)*. These data, which suggest that instructions can modulate the self-administration of orally delivered nicotine, are in keeping with the well-known fact that physicians control their patients' use of narcotics, sedatives, and stimulants.

Physical Dependence Potential of Smokeless Tobacco

Hatsukami and coworkers, at the University of Minnesota, studied neuroadaptation (physiologic dependence) in smokeless tobacco users *(106)*. All 16 subjects in the study used moist snuff and no other nicotine-delivering product. Measures of mood, feeling, behavior, and physiologic function were compared at baseline and during abstinence. Subjects showed significant signs and symptoms of nicotine withdrawal as measured by decreased resting pulse, attenuated orthostatic pulse changes, and increases in tobacco seeking ("craving"), eating, sleep disruptions, and confusion.

A study with nicotine gum showed orally delivered nicotine may cause physical dependence *(107)*. The subjects that were tested had been treated for tobacco dependence with nicotine gum that they used on a daily basis for at least 1 month. Eight subjects were then tested over the course of 4 weeks. They were given nicotine-containing gum during the first and fourth weeks; during the second and third weeks, they received nicotine gum for 1 week and placebo gum for the other. During the week that placebo gum was presented, seven subjects showed signs and symptoms of withdrawal, and two subjects relapsed to smoking or nicotine-containing gum. This study confirms that orally given nicotine has the potential to produce physical dependence. These findings were most recently confirmed by another study that showed development of physical dependence to nicotine gum in patients treated for tobacco dependence *(108)*.

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* Self-administration took place at an average rate of 7.4 pieces compared to an average of 1.2 pieces of placebo gum per day.

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PHYSIOLOGIC AND PATHOGENIC EFFECTS OF NICOTINE AND SMOKELESS TOBACCO

The user of smokeless tobacco is systematically exposed to significant amounts of nicotine, a potent multisystem pharmacologic agent. This chapter addresses the physiologic effects of nicotine upon the cardiovascular, nervous, and endocrine systems and the possible roles of nicotine in the pathogenesis of a variety of diseases.

Nicotine is described in pharmacology textbooks as a stimulant of autonomic ganglia and skeletal neuromuscular junctions (i.e., nicotinic muscarinic receptors). However, in vivo the actions of nicotine are far more complex depending on the dose, target organ, prevalent autonomic tone, and previous exposure history (tolerance) *(1,2)*. For purposes of this review, the focus is on the effects of nicotine in humans. Where human data are lacking and animal studies provide important information about physiologic effects, those studies are also discussed.

Most data on the actions of nicotine in humans derive from studies of the effects of cigarette smoking, comparing cigarettes with and without nicotine, and studies of the effects of intravenous nicotine. These studies provide the basis for our understanding of the human pharmacology of nicotine. However, as noted previously, actions of nicotine from smokeless tobacco and nicotine via inhalation or intravenous infusion may differ.

Physiologic Effects of Nicotine

Cardiovascular System

The predominant cardiovascular actions of nicotine result from activation of the sympathetic nervous system. Smoking a cigarette increases the heart rate (10 to 20 BPM), blood pressure (5 to 10 mmHg), cardiac stroke volume and output, and coronary blood flow *(3-5)*. Smoking may have different effects in smokers with coronary heart disease. It may reduce left ventricular contractility and cardiac output *(6)*, effects that are believed to be related to myocardial ischemia due to smoking-mediated tachycardia and the effects of carbon monoxide. Coronary blood flow may also decrease after smoking, which possibly is related to a nicotine-mediated increase in coronary vascular resistance *(7,8)*. Smoking, or nicotine intake, causes cutaneous vasoconstriction that is associated with a decrease in skin temperature, systemic venoconstriction, and increased muscle blood flow *(9-11)*.

Smoking results in increased circulating concentrations of norepinephrine, consistent with neural adrenergic stimulation, and epinephrine, indicating adrenal medullary stimulation *(3)*. Circulating free fatty acids, glycerol, and lactate concentrations increase. Cardiovascular and metabolic effects are prevented by combined alpha and beta adrenergic blockade, which indicates that the cardiovascular effects of cigarette smoking are mediated by activation of the sympathetic nervous system. Smoking-induced reduction in skin blood flow also can be antagonized by a vascular vasopressin antagonist, which suggests a role for vasopressin in mediating some cardiovascular responses *(2)*.

The cardiovascular effects of oral snuff have been examined systematically in only one study *(13)*. Changes in heart rate and blood pressure that are similar in magnitude to those of cigarette smoking were observed. However, the time course appears to be slower than the response to cigarette smoking, with maximum effects observed at 5 to 10 minutes after a dose of oral tobacco. Similar findings, along with increased myocardial contractility and coronary, femoral, and renal blood flow, were also noted in anesthetized dogs after the administration of oral tobacco *(13)*. Thus it appears that single doses of smokeless tobacco can produce hemodynamic effects that are similar to those of cigarette smoking. Whether such changes are sustained throughout the day with repeated daily doses remains to be established.

Central Nervous System

Although smokers give different explanations for why they smoke, most agree that smoking produces arousal, particularly with the first few cigarettes of the day, as well as relaxation, especially in stressful situations *(14)*. Desynchronization, decreased alpha and theta activity, and increased alpha frequency that is consistent with arousal are the usual electroencephalographic responses to cigarette smoking *(15,16)*. These effects are blocked by mecamylamine, a centrally active nicotinic receptor antagonist, which indicates a role for nicotinic cholinergic receptor activation *(17)*. Tobacco abstinence is associated with effects that are opposite those of smoking, namely, increased alpha power and reduced alpha frequency *(15,18)*.

Endocrine System

Cigarette smoking and nicotine have been reported to increase circulating levels of catecholamines, vasopressin, growth hormone, cortisol, ACTH, and endorphins *(3,19,20)*.

Nicotine inhibits the synthesis of prostacyclin in rabbit aorta and human peripheral veins and the hypoxia-induced release of prostacyclin from rabbit hearts *(21)*. Cigarette smoking has been reported to decrease the urinary excretion of prostacyclin metabolites in humans, which supports the prediction from animal studies *(22)*. Prostacyclin has antiaggregatory and vasodilating actions that are believed to play a homeostatic role in preventing vascular thrombosis.

Nicotine, Smokeless Tobacco, and Human Diseases

As attested to in the Surgeon General's reports since 1964, smoking is a major risk factor for coronary and peripheral vascular disease, cancer, chronic obstructive lung disease, peptic ulcer disease, and reproductive disturbances, including prematurity. Tobacco smoke is a complex mixture of chemicals, including carbon monoxide, many of which are believed to contribute to human disease. Smokeless tobacco likewise exposes users to a number of chemicals, particularly nicotine. Nicotine may play a contributory or supportive role in the pathogenesis of many smoking-related diseases. That nicotine causes human disease de novo has not been proven; however, its potential health consequences deserve serious consideration. More direct data on its causal role are needed.

Coronary and Peripheral Vascular Disease

Nicotine may contribute to atherosclerotic disease by actions on lipid metabolism, coagulation, and hemodynamic effects. Compared to nonsmokers, cigarette smokers have elevated levels of low density (LDL) and very low density lipoproteins (VLDL) and reduced levels of high density lipoproteins (HDL). This profile is associated with an increased risk of atherosclerosis *(23)*. It is hypothesized that nicotine, by releasing free fatty acids, increases the synthesis of triglycerides and VLDL by the liver, which in turn results in decreased HDL production.

In most studies, the blood of smokers is shown to coagulate more easily *(24)*, platelets are found to be more reactive, and platelet survival is shortened when compared to nonsmokers *(25)*. Thrombosis is believed to play a role that promotes the growth of vascular endothelial cells that contribute to the atherosclerotic plaque. The importance of nicotine as a determinant of platelet hyperreactivity is supported by a study that shows an apparent relationship between nicotine concentrations after smoking different brands of cigarettes and platelet aggregation response *(26)*. Nicotine may affect platelets by releasing epinephrine, which is known to enhance platelet reactivity; by inhibiting prostacyclin, an antiaggregatory hormone that is secreted by endothelial cells; or perhaps directly. Finally, by increasing the heart rate and cardiac output, nicotine increases blood turbulence and may promote endothelial injury. Although several potential mechanisms for promoting atherogenesis have been considered, nicotine has not yet been demonstrated to accelerate atherosclerosis in experimental animals.

Nicotine may play a role in causing acute coronary events. Myocardial infarction can occur with one or more of three precipitants: thrombosis, excessive oxygen and substrate demand, and coronary spasm. Nicotine can promote thrombosis as discussed previously. Nicotine increases the heart rate and blood pressure and, therefore, myocardial oxygen consumption. Coronary blood flow increases in a healthy person to meet the increased demand. In the presence of coronary heart disease, ischemia may develop and myocardial dysfunction may occur. Nicotine may induce coronary spasm by sympathomimetic actions or by the inhibition of prostacyclin. Coronary spasm has recently been reported to occur during cigarette smoking *(27)*. All of the above may contribute to the precipitation of acute myocardial infarction in a person with preexisting coronary atherosclerosis.

Cigarette smoke exposure decreases the ventricular fibrillation threshold after experimental myocardial infarction in dogs *(28)*. How much of this effect is due to nicotine and how much is due to carbon monoxide have not been established. Sudden cardiac death in smokers might result from ischemia, as discussed above, combined with the arrhythmogenic effect of increased circulating catecholamines.

Hypertension

Cigarette smoking has not been associated with an increased prevalence of hypertension. However, a recent preliminary report suggested higher blood pressure in young men who used smokeless tobacco compared to cigarette smokers or nonsmokers *(29)*. Smokers who have essential hypertension experience an accelerated progression of vascular and renal disease. Nicotine may contribute to such a process by producing vasoconstriction or enhancing coagulation. There also may be other interactions with hypertensive disease. For example, a patient with a pheochromocytoma developed paroxysmal hypertension and angina pectoris following the use of oral snuff *(30)*. In a controlled situation, blood pressure was recorded to increase from 110/70 to 300/103 with a heart rate increase from 70 to 110 within 10 minutes after the use of oral snuff. Rechallenge after surgery for the pheochromocytoma revealed only the usual blood pressure increase.

Peptic Ulcer Disease

Smoking is strongly related to the prevalence of peptic ulcer disease, and failure to stop smoking is the major predictor of failure to respond to ulcer therapy *(31)*. Smoking decreases pancreatic fluid and bicarbonate secretion that result in greater and more prolonged acidity of gastric fluid of the duodenal bulb *(32)*. Similar effects after the infusion of nicotine have been reported in animals *(33)*. The swallowing of tobacco juice that contains large concentrations of nicotine may conceivably have local effects and therefore elicit added concern for the use of smokeless tobacco.

Pregnancy

Smoking is a major risk factor for low birth weight and, consequently, fetal morbidity and mortality *(34)*. Tobacco smoke may influence the fetus either through alterations in maternal physiology that limit the nutrient flow to the fetus or by the transplacental passage of smoke components that have direct effects on the fetus. The factors that are considered most likely to affect the fetus are carbon monoxide and nicotine. Carbon monoxide inhalation has been shown to increase carboxyhemoglobin in both maternal and fetal blood that possibly limits oxygen supply to the fetus *(35)*. However, while newborn infants of smoking mothers have higher concentrations of carboxyhemoglobin than do neonates of nonsmokers, there are only trivial differences in hemoglobin concentrations, hematocrit, and various characteristics of hemoglobin *(36)*. Thus it is difficult to explain an adverse effect that is based on chronic hypoxia due to carbon monoxide in tobacco smoke. It is more likely that nicotine is important in causing adverse effects.

The effects of nicotine on the fetus may include a reduction of uterine blood flow or a direct effect on fetal function *(37,38)*. The presence of nicotine and its principal metabolites has been demonstrated in the umbilical cord blood and urine of newborn infants of smoking mothers, as well as in amniotic fluid, indicating transplacental passage *(39)*.

Nonnicotine-Related Adverse Metabolic Consequences

Certain brands of chewing tobacco and snuff contain glycyrrhizinic acid, which is also an ingredient of licorice. Glycyrrhizinic acid has potent mineralocorticoid hormone activity that can result in potassium wasting. Two patients who were heavy users of oral smokeless tobacco developed severe hypokalemia with muscle weakness (and in one case, evidence of muscle breakdown) that apparently was due to the ingestion of large amounts of this substance *(40)*. Smokeless tobacco also contains large amounts of sodium *(41)* that, if swallowed, may aggravate hypertension or cardiac failure.

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CONCLUSIONS

1. The use of smokeless tobacco products can lead to nicotine dependence or addiction.
2. An examination of the pharmacokinetics of nicotine (i.e., nicotine absorption, distribution, and elimination) resulting from smoking and smokeless tobacco use indicates that the magnitude of nicotine exposure is similar for both.
3. Despite the complexities of tobacco smoke self-administration, systematic analysis has confirmed that the resulting addiction is similar to that produced and maintained by other addictive drugs in both humans and animals. Animals can learn to discriminate nicotine from other substances because of its effects on the central nervous system. These effects are related to the dose and rate of administration, as is also the case with other drugs of abuse.
4. It has been shown that nicotine functions as a reinforcer under a variety of conditions. It has been confirmed that nicotine can function in all of the capacities that characterize a drug with a liability to widespread abuse. Additionally, as is the case with most other drugs of abuse, nicotine produces effects in the user that are considered desirable to the user. These effects are caused by the nicotine and not simply by the vehicle of delivery (tobacco or tobacco smoke).
5. Nicotine is similar on all critical measures to prototypic drugs of abuse such as morphine and cocaine. The methods and criteria used to establish these similarities are identical to those used for other drugs suspected of having the potential to produce abuse and physiologic dependence. Specifically, nicotine is psycho-

active, producing transient dose-related changes in mood and feeling. It is a euphoriant that produces dose-related increases in scores on standard measures of euphoria. It is a reinforcer (or reward) in both human and animal intravenous self-administration paradigms, functioning as do other drugs of abuse. Additionally, nicotine through smoking produces the same effects, and it causes neuroadaptation leading to tolerance and physiologic dependence. Taken together, these results confirm the hypothesis that the role of nicotine in the compulsive use of tobacco is the same as the role of morphine in the compulsive use of opium derivatives or of cocaine in the compulsive use of coca derivatives.

6. The evidence that smokeless tobacco is addicting includes the pharmacologic role of nicotine dose in regulating tobacco intake; the commonalities between nicotine and other prototypic dependence-producing substances; the abuse liability and dependence potential of nicotine; and the direct, albeit limited at present, evidence that orally delivered nicotine retains the characteristics of an addictive drug.

7. Several other characteristics of tobacco products in general, including smokeless tobacco, may function to enhance further the number of persons who are afflicted by nicotine dependence: nicotine-delivering products are widely available and relatively inexpensive; and the self-administration of such products is legal, relatively well tolerated by society, and produces minimal disruption to cognitive and behavioral performance. Nicotine produces a variety of individual-specific therapeutic actions such as mood and performance enhancement; and the brief effects of nicotine ensure that conditioning occurs, because the behavior is associated with numerous concomitant environmental stimuli.

8. All commonly marketed and consumed smokeless tobacco products contain substantial quantities of nicotine; the nicotine is delivered to the central nervous system in addicting quantities when used in the fashion that each form is commonly used (or as recommended in smokeless tobacco marketing campaigns).

9. Since the exposure to nicotine from smokeless tobacco is similar in magnitude to nicotine exposure from cigarette smoking, the health consequences of smoking that are caused by nicotine also would be expected to be hazards of smokeless tobacco use. Areas of particular concern in which nicotine may play a contributory or supportive role in the pathogenesis of disease include coronary artery and peripheral vascular disease, hypertension, peptic ulcer disease, and fetal mortality and morbidity.

RESEARCH NEEDS

Available data clearly support the view that nicotine produces behavioral and physiologic dependence and has effects on all critical dimensions exemplified by a drug with a profile of high abuse liability. Nevertheless, the resolution of several questions is essential. These questions revolve around the relationships between the several forms of tobacco use. They parallel and have commonalities with important issues in other forms of drug abuse (e.g., cocaine). There are several major research areas that could provide data of potential public health significance.

The first area of research is the relationship between the rate of nicotine administration and abuse liability. Existing data suggest that the slowest commercially available nicotine-releasing preparation, nicotine gum, has a lower abuse liability than the fastest commercially available nicotine-releasing preparation, cigarettes. These facts further suggest the possibility that there might be quantifiable differences in abuse liability among tobacco product forms.

The second area of research importance involves the relationship between the initiation of one form of tobacco use, e.g., smokeless tobacco, and the use of other forms of tobacco, e.g., cigarettes. The relationships between common forms of tobacco use, the extent to which they are interchangeable, and the possibility that the use of one form of tobacco leads to the use of another need examination.

A third area of specific importance relates to the extent to which tobacco use, with its implicit acceptance, encourages other drug use. A related question is the extent to which exposure to drug effects, both neurologic and behavioral, modifies subsequent drug responses or establishes the conditions for other equally harmful drugs to become reinforcers. These issues follow from the observations that cigarette use is a major correlate (possibly a "stepping stone") of other kinds of drug dependence and that regular tobacco use generally leads to other forms of drug addiction.

A fourth area of research is prevention and treatment. Recent surveys indicate that youth attribute negligible risk to smokeless tobacco products, suggesting the possible need for education-based prevention approaches. Regarding treatment, it is plausible that nicotine gum treatment could be of even greater relative utility for smokeless tobacco users than for cigarette smokers because of the more similar pharmacokinetic profiles of smokeless tobacco- and gum-delivered nicotine compared to cigarette smoke-delivered nicotine.

The absorption and distribution characteristics of nicotine with the use of smokeless tobacco may differ from those of cigarette smoking. The pharmacodynamic and pharmacologic consequences of such differences may be important but require additional future research. Further studies to define more precisely the role of nicotine and of smokeless tobacco in the causation of diseases other than those that involve the oral cavity are clearly needed. Specifically, research is needed to:

- Determine nicotine blood levels and time course in various populations of smokeless tobacco users, including established users.
- Determine the cardiovascular, hormonal, and metabolic effects of smokeless tobacco when used in a regular fashion throughout the day.
- Determine the influence of the rate of absorption of nicotine on the effects from smoking cigarettes and the use of smokeless tobacco.
- Using experimental studies, determine the effects of smokeless tobacco in users of different ages and high-risk status (i.e., patients with hypertension, coronary heart disease, peripheral vascular disease, and peptic ulcer).
- Using epidemiologic studies, determine the risk potential of the regular use of smokeless tobacco on the development of diseases such as coronary heart disease, peptic ulcer, and complications of pregnancy.

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